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CONTENTS

Rolla Kent Beattie, 1875-1960 — John A. Stevenson 135

Attraction of parasitic nematodes by plant root emanations — David R. Viglierchio 136

A synergistic Fusarium-nematode interaction in carnations — A. F. Schindler, Robert N. Stewart, and Peter Semeniuk 143

Influence of Tylenchulus semipenetrans on the growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios — S. D. van Gundy and J. P. Martin 146

Effects of selected Cytospora isolates from stone fruits on certain stone fruit varieties — A. W. Helton and D. E. Konicek 152

Strains of Pseudomonas solanacearum differing in pathogenicity to tobacco and peanut — Arthur Kelman and L. H. Person 158

Complementary isolates of Phytophthora palmivora from cacao and rubber, and their taxonomy — P. D. Turner 161

The use of Fusarium culture filtrate media in the isolation of Fusaria from soil — J. R. Parmeter, Jr., and J. R. Hood 164

Influence of soil, temperature, isolates, and method of inoculation on resistance of mint to Verticillium wilt — S. Z. Berry and C. A. Thomas 169

Resistance to the wildfire disease in relation to the gene-chromosome set ratio in tobacco — J. H. Smiley and G. W. Stokes 174

Inoculating barley seedlings with Ustilago nuda and wheat seedlings with U. tritici — Thomas Kavanagh 175

Toxin production by Helminthosporium victoriae on synthetic media containing different nitrogen sources — Robert W. Berry and Maurice C. Futrell 177

The role of pectic substances in chemically induced resistance to Fusarium wilt of tomato — L. V. Edgington, M. E. Corden, and A. E. Dimond 179

Rod-shaped particles associated with virosis of spruce — M. Cech, O. Kralik, and C. Blattny 183

Artificial inoculation of longleaf pine with Scirrhia acicola — Glenn A. Snow 186

Temperature in relation to loose smut in barley and wheat — Thomas Kavanagh 189

Phytopathological Notes

Soil transmission of tobacco ringspot virus — James W. Hendrix 194

Effect of auxins on virus-induced leaf abscission — Joan Gwyneth Dyson and M. Chessin 195

Chemotaxis of zoospores of Phytophthora parasitica var. nicotianae by plant roots and certain chemical solutions — P. D. Dukes and J. L. Apple 195

Corn gray spot caused by Piricularia grisea — A. G. Bailey and C. van Eijnatten 197

Purification and electron microscopy of the peach yellow bud mosaic virus — M. Weintraub 198

Serological detection of barley stripe mosaic virus in single seeds and dehydrated leaf tissues — H. A. Scott 200

A technique for inoculating leaves with root-knot nematodes — N. T. Powell and E. L. Moore 201

Relation of temperature to reaction type of Puccinia coronata on certain oat varieties — David E. Zimmer and John F. Schafer 202

Sporulation of Pyrenophora bromi in culture — J. F. Carter and J. G. Dickson 204

A weed host of Xanthomonas phaseoli var. sojense — John P. Jones 206

The President's Column 207

News 208

Book Listings 168, 185, 188

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ROLLA KENT BEATTIE, 1875—1960

John A. Stevenson

Rolla Kent Beattie, or R. Kent Beattie, as he preferred to be known, botanist and plant pathologist, died in Bethesda, Maryland, June 2, 1960. Mr. Beattie was born in Ashland, Ohio, on January 14, 1875, the son of James A. and Margaret (Benton) Beattie. He received the A.B. degree from Cotner University (Lincoln, Nebraska) in 1895, and the B.S. and M.A. degrees from the University of Nebraska, respectively in 1896 and 1898. At the latter institution he, of course, came under the inspiring influence of Charles E. Bessey, one of the most eminent botanical teachers of the time, and he ever remained an ardent disciple of the Besseyan school of botany. Among his classmates were Ernst Bessey, C. L. Shear, G. G. Hedgcock, and F. E. Clements, all in due time to win fame in the botanical world.

Beattie taught science in the Leadville, Colorado, and Cheyenne, Wyoming, high schools for two years before becoming instructor, in 1899, in the botany department of Washington State College, Pullman. There, in collaboration with C. V. Piper, three floristic studies were prepared: *The Flora of the Palouse Region*, *Flora of Southeastern Washington and Adjacent Idaho*, and *Flora of the Northwest Coast*. He succeeded Piper as head of the department and botanist in the Agricultural Experiment Station in 1903. In addition to carrying a heavy teaching load, he collaborated with A. L. Melander, the entomologist, in pioneer studies of lime-sulfur as a fungicide, particularly for the control of apple scab. He studied other plant diseases prevalent in the area and experimented with the penetration system of orchard spraying, again in cooperation with Melander.

His life-long friend and fellow Bessey student Haven Metcalf brought him to Washington in 1912 to take part in a campaign, under way in the Bureau of Plant Industry, against the then recently introduced chestnut blight fungus. In this work he speedily demonstrated his ability as an organizer and administrator of such emergency phytopathological operations.

In 1914 his skill in this field led to his transfer to the Federal Horticultural Board, newly set up under the authority of the Plant Quarantine Act of 1912. There he took an active part in establishing plant inspection procedures and administering the several specific quarantines promulgated under the provisions of the act, in particular the highly controversial Quarantine 37, which regulated the entry of nursery stock from foreign countries. For a time he also directed the Plant Disease Survey for the Bureau of Plant Industry, but soon turned its development over to Dr. George R. Lyman, who had come to Washington from Dartmouth College as Beattie's associate in both the quarantine and plant disease survey activities.

Metcalf lured Beattie back into the Bureau of Plant Industry in 1927, with the intriguing and still unsolved problem of chestnut blight. On this assignment he spent over three years in an intensive study of the genus *Castanea* and its relatives in Japan, China, and other Oriental countries. He made extensive

collections of nuts of promising types for introduction into America, studied the blight in its Oriental setting, and prepared abundant herbarium specimens of both hosts and fungi to document his work. As an important side line he obtained from Japanese friends an extensive series of Kurume azaleas whose introduction into America has been hailed as a major contribution to the development of azalea varieties in this country. Many of the Oriental chestnut varieties gathered by him are growing in this country or have proved useful in the breeding program that is the basis of the current attack on the problem of replacing the American chestnut.

Following his work in the Orient, Beattie was placed in charge of the Department's program aimed at controlling introduced forest tree diseases. He organized the attack on the Dutch elm disease, including the scouting and control programs, work that was later turned over to the Bureau of Entomology and Plant Quarantine for operation. Until his retirement he directed research on this disease and other imported tree diseases, including larch canker and fir needle cast. Retiring in 1945, he took up a study of the life and work of David Douglas, a pioneer plant explorer in the Pacific Northwest in whom he had long been interested. Ill health prevented completion and publication of the results of his studies.

Mr. Beattie married Rose Mozee at Geneva, Nebraska, in 1900. She died in 1950. He leaves a daughter, Mrs. Lois Taylor, of Potomac, Maryland, a granddaughter, and a great-grandson. He was a life member of the American Phytopathological Society and during his active career belonged to a number of other technical societies.

ATTRACTION OF PARASITIC NEMATODES BY PLANT ROOT EMANATIONS

David R. Viglierchio

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Accepted for publication August 12, 1960.

SUMMARY

The attraction of parasitic nematodes (*Meloidogyne hapla, M. incognita acrita,* and *Heterodera schachtii*) by host plant roots was demonstrated with growing plants in sand and soil by 2 methods. Nematodes were observed to accumulate at a membrane separating them from roots. The attracting stimulus from tomato is a dialyzable diffusing agent or agents effective at a range of at least 10 mm. *H. schachtii* larvae were weakly attracted to tomato but repelled by Kanota oat roots. *M. hapla larvae* were strongly attracted to non-host Kanota oat roots, and both strongly repelled and weakly attracted to Merced rye roots. The positive correlation for plants of strength of attraction with efficiency as a host for parasitic nematodes is unwarranted.

Introduction.—Workers interested in the attraction of plant-parasitic nematodes by plants have generally employed special conditions for their studies. Wieser (13, 14), Lownsbery and Viglierchio (7, 8), and Wallace (11) used germinating seeds, excised or whole, in small dishes or funnels; Bird (2) used excised roots and leachings from plants grown hydroponically; Kühn (5) used tuber pieces. Since physiological reactions are not identical in germinating seeds and in plants, and excised roots or tuber sections are artifacts manifesting confounding reactions, e.g., wounding, it would be of interest to compare nematode responses to germinating seeds with responses to growing plants.

Materials and methods.—*Lateral migration experiments.*—Aluminum dishes were constructed of extruded channel aluminum (external dimensions 127 × 44 mm) simply by cutting off strips of channel. A strip about 150 mm long was used for growing plants, and a shorter strip (70–80 mm long) was used for the nematodes. The channel sections were cut and sanded so that the ends (normal to the long axis of the extruded channel) would butt together squarely. Aluminum plates (127 × 44 × 15 mm) were cut to cap the ends of the channel pieces. The channel interiors and plates were coated with asphaltum paint to avoid aluminum toxicity problems. The short strips of channel to serve as the nematode dishes were marked off in 10 mm intervals to facilitate the cutting of sections of sand or soil 10 mm thick.

A cutting guide was constructed by bolting two strips of rectangular aluminum bar (180 × 20 × 12 mm) together, with a spacer between them sufficiently thick to permit a spatula to slide along the slot between the bars. Alignment of the slot with the 10-mm markings inscribed on the edges of the nematode channel strip permitted the cutting of sand or soil sections with an error of less than 10%.

Seeds of host plants were planted to a depth of 3 cm in the larger channel dishes filled with quartz sand (grain size minus 30 mesh, with about 5% minus 150 mesh) or sandy loam soil. The germinated plants, thinned to about 100 seedlings per dish, were watered with mineral nutrient solution until they were about 150–200 mm tall (12).

Then the end plates were removed from the plant dish, and the butt ends were wiped free of sand or soil and covered with a coating of lanolin, as were the ends of the nematode dishes. A nematode dish was butted to each end of the plant dish with a section of dialysis membrane between the dishes; end plates were placed at the extreme ends of the series (the ends of the nematode dishes away from the plants). The membrane strips were 42.6-mm-diameter dialysis tubing (viscose-process regenerated cellulose manufactured by Visking Corporation), average pore size 24 angstroms, cut to size. The lanolin prevented leakage of water at the joints of the dish assembly. The dishes, kept together by a spring-chain arrangement encircling the whole assembly, were leveled for the next step. A suspension of 1–2-day-old larvae (10,000–60,000, depending on the number available), well mixed in a predetermined volume of water, were poured into the nematode dish, and sandy loam soil or washed quartz sand was sprinkled into the dish as evenly as possible with the aid of screens. The volume of final nematode suspension was precalculated so that when sufficient sand or soil had been added to give a well-wet matrix, the level of sand or soil corresponded to the level in the plant dish (Fig. 1).

The plant dish was wetted to approximate the wetness of the nematode dish, and then the assembly was placed in a high-humidity chamber to reduce transpiration, water movement, and water loss. After 48 hours of incubation at room temperature under 6 warm white fluorescent light tubes about 100 mm above the plants, the assemblies were removed from the humidity chamber and sand or soil was sprinkled upon the nematode dishes to firm them up for the cutting of 10-mm sections from the membrane outward. Each section of soil or sand was placed on a Baermann funnel for 3 days before the larvae were collected and counted.

After the experiments with nematodes in sand followed by nematodes in soil, each plant dish was leached by adding distilled water repeatedly until about a liter of distilled water had percolated through the soil or sand and drained away. The intent of the leaching was to reduce the amount of soluble materials (salts and root exudate). After 24 hours the migration experiments with nematodes in washed sand and soil were repeated as previously described.

Sublateral migration experiments.—In these tests a longer piece of channel aluminum (1300 mm) was

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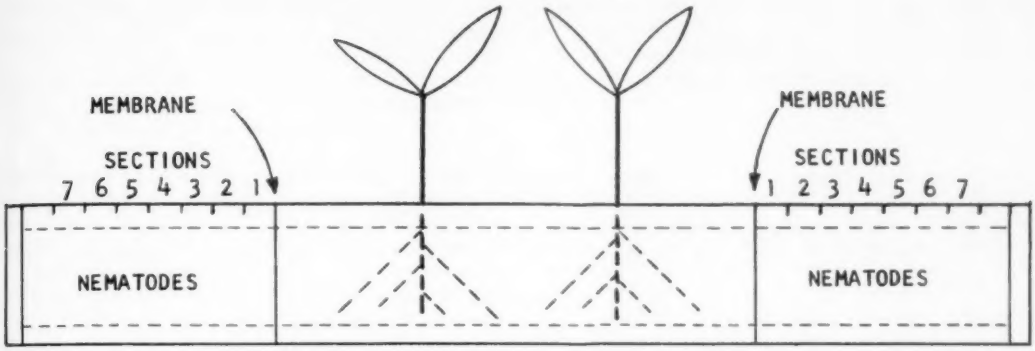


Fig. 1.—Schematic diagram of the apparatus used in the lateral migration experiments. The zone between the dotted lines indicates the soil or sand matrix.

similarly cut and painted. The length of channel was then inscribed at 10-mm intervals.

Sublateral migration experiments with the long channel made use of techniques similar to those already described. The dish was leveled and a suspension of nematode larvae (150,000–300,000, depending on the number available) well mixed in a predetermined volume of water (to give about a 10-mm-layer of sand subsequently) was poured into it. Sufficient sand was sprinkled into the nematode suspension to provide a firm bed but yet remain well wet. A wet dialysis membrane (42.6-mm-diameter tubing slit longitudinally) was placed on the nematode-infested sand. The edges of the membrane were sealed against the channel wall by water adhesion. The tray was then divided into five plant sections, each 50 mm wide, with 200 mm between sections and 120 mm from the end plates. Seeds previously germinated (1–3 days old, depending upon the kind) on wet filter paper were distributed on the membrane in these sections (50–100 per section). Cellulose acetate (1.5 mm thick) dividers were cut so as to fit tightly against the membrane on the bottom and channel walls and inserted between the plant and plant-free zones to retain the developing root systems to the plant section. More water was added to the wet membrane, and sufficient sand sprinkled on all the tray to a depth just covering the germinating seed (Fig. 2). The tray was set in a humidity chamber in the greenhouse or under fluorescent lights in the laboratory. The sand was kept wet by misting with water as needed. After 5–7 days the tray was removed from the humidity chamber, the membrane, sand and plants were discarded, and the sand with nematodes was cut into 10-mm sections as described and set up on Baermann funnels for 3 days before the larvae were collected for counting.

Results and discussion.—*Good host plants.*—The method devised in this work to establish an even distribution of nematodes in soil or sand was effective. The control curve with no plants (as indicated in Fig. 5) shows that the distribution was sufficiently uniform for the purposes of these experiments.

The distribution of nematode larvae after 48-hour lateral exposure to host plants is shown in Fig. 3–5. For practical considerations (varying dish sizes, numbers of nematodes, etc.) the number of animals in each 10-mm section of sand or soil is shown as a percentage of the total recovered from that dish. Each point in the curves is the average of 4–8 replicates. Total recovery varied from 30–60%, depending on the number initially added. The greatest loss of animals was probably due to discarded strips of sand or soil in oversize dishes and overloaded Baermann funnels. The Baermann funnel technique is inefficient in terms of quantitative recoveries except under special conditions; it was used for its principal advantages—simplicity, convenience, and selectivity of live animals free of debris.

The curves indicate that *Meloidogyne hapla* and *M. incognita acrita* can accumulate in the 10-mm section near the membrane separating tomato seedling roots and nematodes. *Heterodera schachtii* can ac-

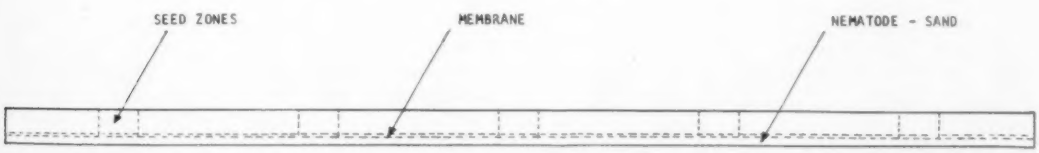
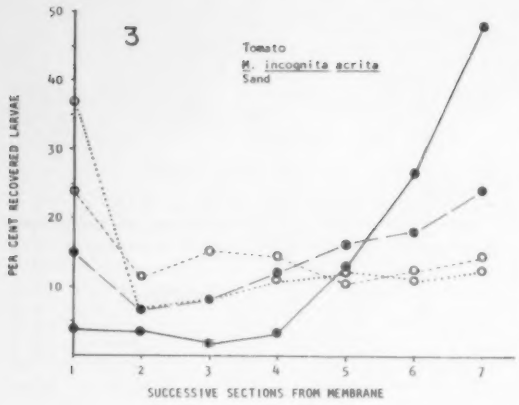
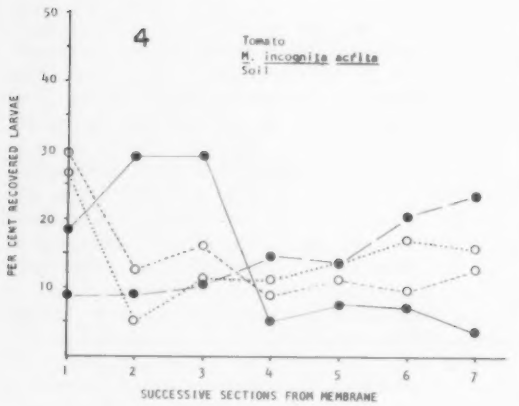


Fig. 2.—Schematic diagram of the apparatus used in the sublateral migration experiments. The zone between the two horizontal dotted lines indicates the layer of sand just covering the germinating seeds above the membrane (lower dotted line).





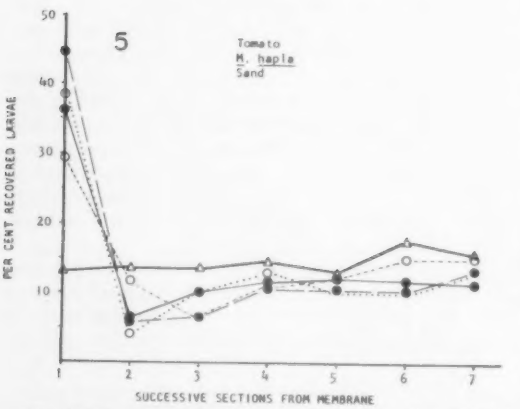


Fig. 3–5.—The curves show the distribution of nematodes after 48-hour lateral exposure to the roots of growing plants. In each figure the plant, the medium in which the plant was grown, and the nematode are indicated. The solid circles, solid lines indicate the results with nematodes in sand and unleached plants; solid circles, long dashed lines indicate the results with nematodes in soil and unleached plants; open circles, short dashed lines indicate nematodes in sand and leached plants; open circles, dotted lines indicate the results with nematodes in soil and leached plants; open triangles, solid line indicate the results (control) with nematodes in the same medium as indicated for the plants as though plants were present.

cumulate similarly near sugar beet seedlings. The nematode response was the same whether the plants were in sand or soil. The curves of unleached tomatoes and *M. incognita acrita* (Fig. 3, 4) were especially selected (the normal curves being similar to those of Fig. 5) to illustrate two processes, repulsion and attraction, which can be operative. The repulsion is probably a result of a high concentration of salts from excess nutrient and root exudation, since leaching provides the normal curve. The unleached sand curve of Fig. 4 shows a maximum, which can be explained by repulsion due to salts and attraction due to roots occurring simultaneously. The salt effect would also explain the observations with plants in sand that the accumulation occurring with nematodes in soil is greater than with nematodes in sand. In this case the salt effect reinforces the attraction of plants.

Soil moisture was not a confounding factor in these experiments, i.e., the distribution curves obtained with moist sand or soil and very wet sand or soil were similar, though total recovery of animals tended to be lower at the lower moisture levels.

It is of interest to observe that some of the animals found in section 1 appear to have come from sections as far removed as 3 and 4. It is apparent that some larvae must have come from sections farther removed than 1 and 2, but it is uncertain whether larvae moved from sections 3 and 4 in response to chemical stimuli or to the lower population density in a local area depleted by the attraction effect. If, between plants and nematodes, two membranes separated by some thickness of sand or soil were inserted instead of the usual membrane, it would be possible to observe the transmission of chemical stimuli over a finite distance. The curves of Fig. 6 indicate that the stimuli are able to penetrate two membranes and 10 mm of sand or soil with little loss of activity. The migration of animals in the original sections 3 and 4 would then be effected both by chemical stimuli from the plants as well as by the local population density gradient set up by the initial larval attraction in section 1. The manifestation of attraction through two membranes confirms the dialyzable nature of the stimulating agent (7, 8, 9).

When 3-day-germinated tomato seeds in sand were substituted in the plant dish for 5-week-old tomato plants in migration experiments, distribution curves similar to those obtained with 5-week-old plants were obtained. Nematode larvae accumulated in the sand section adjacent to the membrane. The ability of tomato to attract root-knot larvae appears not to be a function of age of plant.

The sublateral migration experiments were conducted to confirm the results of lateral migration by a somewhat different method. The distribution of *M. incognita acrita* with Rutgers tomato (there appeared to be no difference between Rutgers and Pearson tomatoes) was similar to that of *M. hapla* and Rutgers tomato shown in Fig. 7. It is obvious that numbers of larvae are greatest under the plant zones where the five high peaks are observed. The fine structure is somewhat questionable; however, there is a tendency toward lesser peaks centrally between plant zones. The

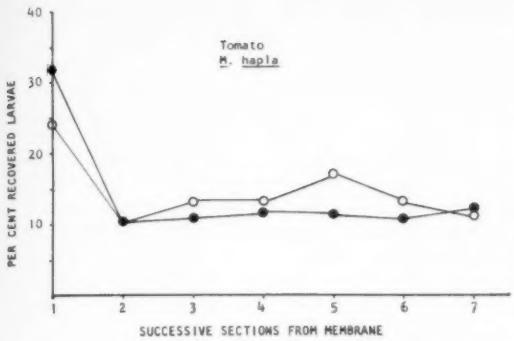


Fig. 6.—Nematode distribution after 60-hour lateral exposure to plant roots through 2 membranes and a 1-cm-thick layer of sand or soil. Filled circles indicate soil matrix throughout (plants, insert, and nematodes); open circles indicate sand matrix throughout (plants, insert, and nematodes).

no-plant zone peaks indicate: a) that attraction is probably of little importance in the 10-cm range, and b) that the curves are not merely nematode survival patterns. The sloping shoulders of some of the plant zone peaks are the result of the escape of roots under the dividers inserted to restrict them.

As one might expect in an unfavorable situation with poor aeration, poor moisture balance, and high temperature (room temperature) for upwards of a week, there was a considerable loss of viability as indicated by recovery of only 10–30% of the initial population. *M. hapla* appeared more sensitive under these conditions than *M. incognita acrita*.

Since the tomatoes at the beginning of the experiment were 3-day-old germinating seeds, they were essentially independent seedlings by the tenth day. It is apparent, then, that attraction in tomatoes is manifested by germinating seeds 3–4 days old (7, 8), very small seedlings 3–10 days old, and larger plants 5–6 weeks old.

Poor and non-host plants.—The response of nematodes to lateral exposure to poor and non-host plant roots is shown in Fig. 8–10, where each point is the average of 4–8 replicates. With *Heterodera schachtii* and Pearson tomato (Fig. 8, 9) there is evidence for attraction, though much less than with *H. schachtii* and sugar beet. The all-soil system (Fig. 9) shows a greater attraction than does the system of plant in sand and nematode in soil. Both repulsion and attraction appear to occur with the systems of all sand and of plant in soil and nematode in sand. The repulsion can be due to salts, some substance in the root exudate, or both.

The Pearson variety of tomato was tested against the population of *H. schachtii* used in these experiments for varietal or racial effects in root penetration. Young tomato and sugar beet seedlings in pots were exposed to known numbers of nematodes; 12 days later the plants were sacrificed and the roots stained with lactophenol–acid fuchsin and examined for larvae. Second- and third-stage larvae were found in large numbers in the sugar beet roots and only occasionally in the tomato roots. The indication that Pearson tomato is a poor host for the sugar beet nematode confirms observations by Golden and Shafer (4).

Lateral tests with Kanota oats and *Meloidogyne incognita acrita* indicated that attraction was somewhat weaker than with tomato. Oats whether in sand or soil, leached or unleached, atracted nematodes in sand.

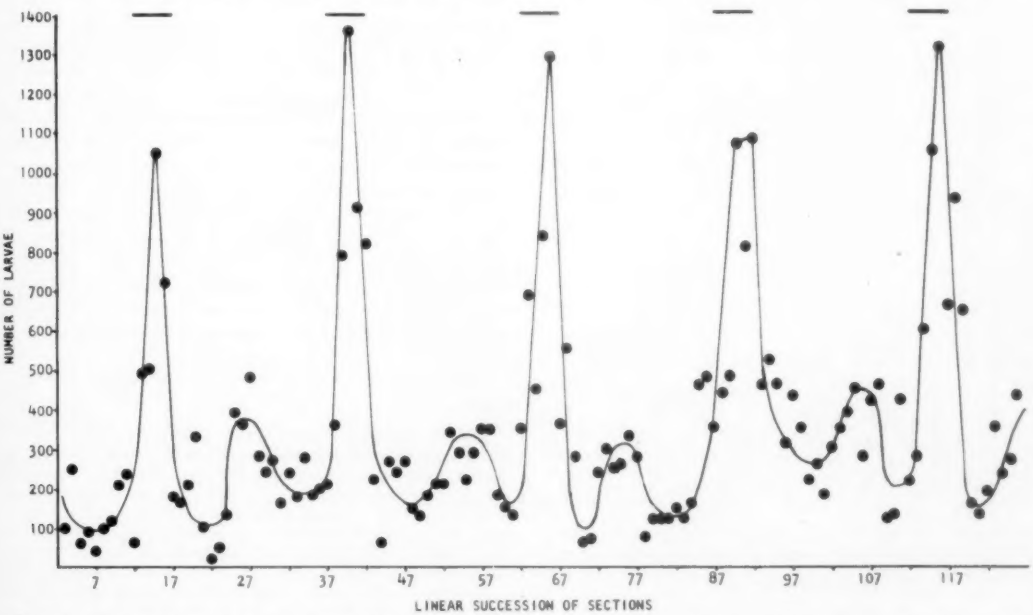
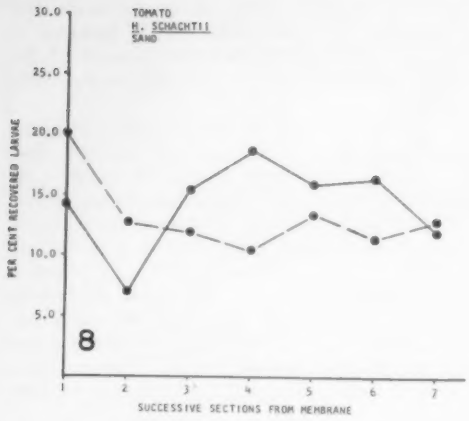
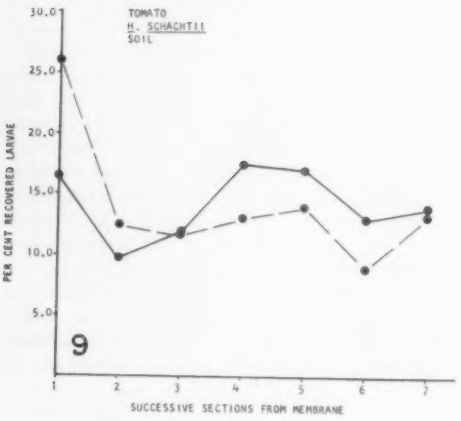
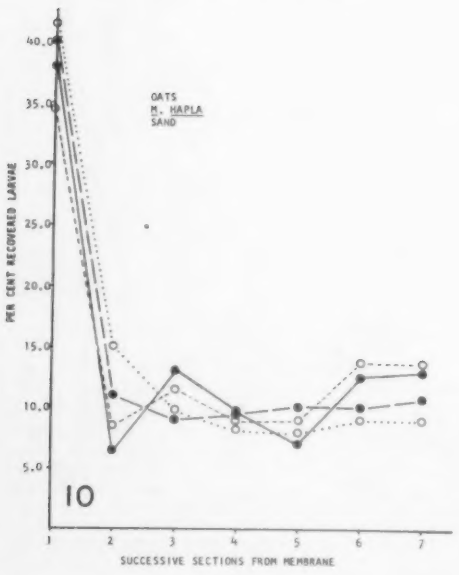


Fig. 7. The distribution of *M. hapla* after 5 days' sublateral exposure to tomato seedling roots. The bars at the top of the figure indicate the plant zones.







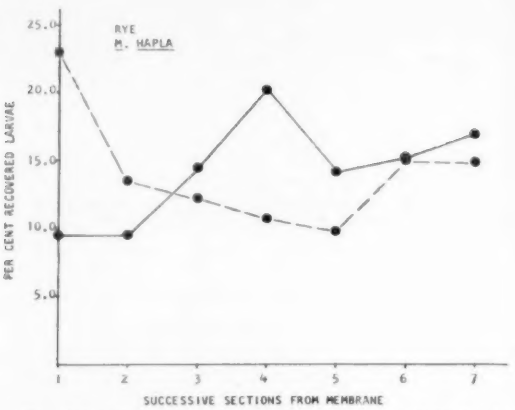


Fig. 11.—The curves show the distribution of *M. hapla* larvae after 48-hour lateral exposure to Merced rye. The solid line indicates the distribution with plants and nematodes in sand; the broken line indicates the distribution with plants and nematodes in soil.

Sublateral experiments with germinating oats and *M. incognita acrita* confirmed the previous conclusions. The typical attraction response, noted with tomato and *M. incognita acrita*, with high plant-zone peaks and lower interplant-zone peaks was obtained. The attraction of *M. incognita acrita* by Kanota oats is independent of plant age.

Kanota oat and Pearson tomato seedlings (70–100 mm tall) were exposed to 2500 second-stage larvae of *M. incognita acrita*; after 3 days the plant roots were stained and examined for nematodes. Kanota oat roots were invaded as heavily as tomato roots. At the beginning of the experiments it was thought that Kanota oats were resistant to *M. incognita acrita*. It would have been more advantageous to have tested some of the varieties used by Crittenden (3) or Allen (1).

Penetration tests with Kanota oats and *H. schachtii* similar to those described for tomato indicated no penetration of roots by infective second-stage larvae under conditions in which sugar beet seedlings suffered massive invasions. These findings suggest that Kanota oats are a very poor host for, or immune to, the sugar beet nematode. Sublateral migration tests with *H. schachtii* and oats indicate repulsion (Fig. 12). Minima exist at plant zones, and maxima at inter-plant zones, as one would expect for repulsion. Whether the lack

Fig. 8–10.—The curves show the distribution of nematodes after 48-hour lateral exposure to the roots of growing poor or non-host plants. In each figure the plant, the medium in which the plant was grown, and the nematode are indicated. The solid circles and lines indicate the results with nematodes in sand and unleached plants; solid circles with long dashed lines indicate nematodes in soil and unleached plants; open circles with short dashed lines indicate nematodes in sand and leached plants; open circles with dotted lines indicate nematodes in soil and leached plants.

of penetration of oats is due to increasing repulsion of *H. schachtii* as it approaches the root is a matter of speculation.

In the case of *H. schachtii* the attractiveness of a plant was found to be correlated with its efficiency as a host, e.g., sugar beet, a good host and strong attraciton; tomato, a poor host and weak attraction; oats, a very poor host or immune plant and repulsion. It was therefore of interest to investigate the attraction response with a different animal. *Meloidogyne hapla* was selected, for the literature (10) indicates that at least two plants, oats and rye, are immune to this animal. Not only was *M. hapla* unable to reproduce but it did not penetrate the roots of either plant. These observations, using the *M. hapla* of these experiments, were substantiated with Kanota oats and Merced rye; tomato under the same conditions suffered massive invasions.

The attraction response of *M. hapla* in lateral exposure to Kanota oats is indicated in Fig. 10. There is strong attraction, comparable to that with tomato, in sand, soil, or either combination, whether leached or unleached. Sublateral tests (Fig. 13) indicate the typical attraction response—high peaks at the plant zones, smaller peaks centrally in the interplant zones. It is apparent, therefore, that Kanota oat attracts

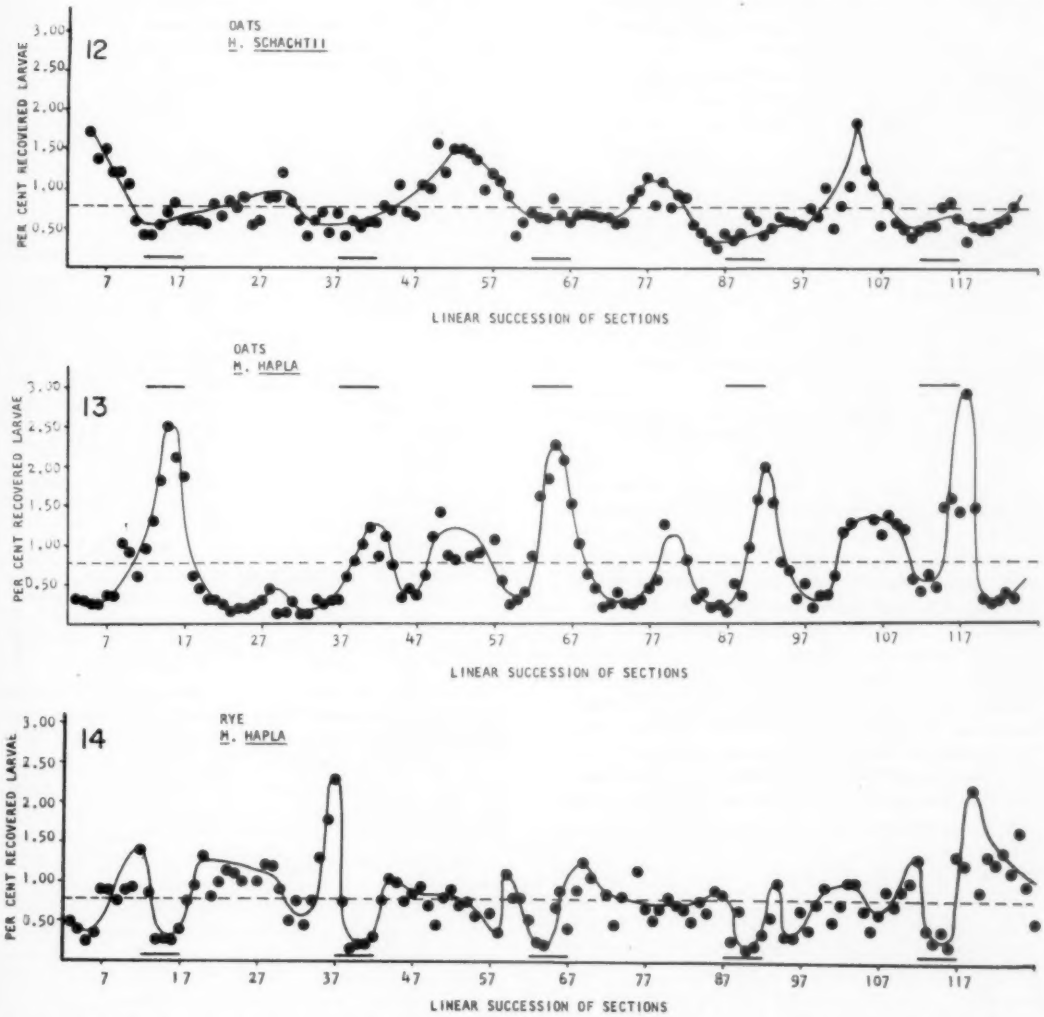


Fig. 12–14.—The curves show the distribution of nematodes upon sublateral exposure to plant roots. The bars represent the width and position of the plant zones. Plants and animals are indicated for each curve. The dotted lines indicate the theoretical average per cent distribution of nematodes. For *M. hapla* the incubation period was 5 days; for other species, 7 days.

M. hapla though it is completely useless to the nematode for nutrition or reproduction.

M. hapla gave different results with Merced rye than with oats. The lateral response (Fig. 11) shows that the predominant effect is repulsion with an all-sand system, and attraction with an all-soil system. It is unlikely that the repulsion is due to excess mineral salts resulting from nutrient watering of the plants. Oats and rye develop good root systems about a week from germination; tomatoes, with which salt repulsion was observed, require on the order of a month. It therefore would appear that the stimuli of attraction and repulsion originate from the roots of the rye seedlings. If this were so, then the soil colloids probably adsorb the stimuli of repulsion, masking weaker stimuli of attraction apparent in the all-soil but not the all-sand system.

There is some evidence for this speculation in the sublateral experiments with rye and *M. hapla* (Fig. 14). The matrix system here was all-sand with no nutrient, and the repulsion response observed with lateral tests is manifested primarily by the low minima centrally located in the plant zones. The maxima on each side of a minima would be the expected accumulation of nematodes moving away from the plant zone. The proximity of these peaks to the plant zones is of importance, for they indicate the presence of two simultaneous effects. In simple repulsion the animals would be expected to move away from the plant zones to accumulate centrally between the plant zones, as with *H. schachtii* and oats (Fig. 12). In contrast, with rye and *M. hapla* the accumulation maxima are very near the plant zone minima. Furthermore, next to the maxima and away from the plant zone in the regions of sections 5, 32, 55, 96, and 106, occur lesser minima (below the theoretical average), indicating a localized depletion of animals. Such a distribution of animals from the plant zone outward—very low, high, low, and intermediate, could be readily explained by the previous suggestion of a nematode response to a root exudate containing both attracting and repelling stimuli.

These naturally occurring stimuli of repulsion or attraction, if chemical, are low-molecular-weight dialyzable substances. Compounds such as glutamic acid, suggested by Bird (2), would fall in this category. The nature of the attractant is certainly not unique. The response is likely effected by several substances or resulting gradients. A mechanism of action of attraction and repulsion that would also explain a simultaneous occurrence is yet a matter of speculation. In any event, correlation of attraction with host preference or specificity is unwarranted. The *apparent* correlation with *H. schachtii* and sugar beet, tomato, and oats may be the result of a fortuitous selection of plants; other plants would need to be tested. The existence of attractive nonhost plants has been demonstrated; the reverse situation of an unattractive host has not yet been observed. It would appear to be only a question of an adequate search for one.—Department of Nematology, University of California, Davis.

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A SYNERGISTIC FUSARIUM-NEMATODE INTERACTION IN CARNATIONS

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SUMMARY

Carnations were inoculated with the parasitic nematodes *Meloidogyne hapla, M. incognita, M. incognita acrita, M. arenaria, M. arenaria thamesi, M. javanica, Helicotylenchus nannus, H. buxophilus,* and the carnation Fusarium wilt fungus, *Fusarium oxysporum* f. *dianthi*. When Fusarium inoculum was applied 1 week, 2 months, or 5 months after root-knot nematode inoculations, there was a synergistic interaction between the two pathogens which greatly increased the severity of the Fusarium wilt disease. This interaction with *Fusarium* was not shown with either *H. nannus* or *H. buxophilus*. Pathogenicity of *M. incognita acrita, M. incognita, M. arenaria thamesi,* and *H. nannus* on carnations was demonstrated. In an experiment where *H. nannus* was pathogenic to carnations it did not interact with *Fusarium*. In contrast, several root-knot species at inoculum levels which caused no injury in the absence of *Fusarium* did interact with *Fusarium* where it was present.

Plant-parasitic nematodes, reported on carnations as early as 1909 (2), may increase the severity of bacterial wilt of carnations (14). Both greenhouse and field trials have demonstrated that several other crops are more severely damaged by Fusarium diseases or exhibit symptoms sooner when nematodes attack the host plants (3, 7, 8, 9, 10, 11). Reynolds and Hanson (12), working with a *Rhizoctonia*–root-knot nematode complex of cotton, stated that the nematodes acted as debilitating factors, affecting plant vigor, weakening the plant, and making it more susceptible to attack by *Rhizoctonia solani* Kuehn. When a survey revealed that plant-parasitic nematodes were present in most carnation greenhouse soils (13), the effect of several species of nematodes on the fusarium wilt of carnations was investigated.

Materials and methods.—All carnation plants were grown from cuttings rooted in a sterile propagating bed. A number of Fusarium wilt susceptible commercial cultivars and Beltsville selections were used in the tests. The four cultures of *Fusarium oxysporum* Schlecht. f. *dianthi* (Prill. & Del.) Snyd. & Hans. used were originally obtained as single-spore isolates from Colorado, New York, and Connecticut by Hood and Stewart (5). The fungus was grown in shake-cultures for 72 hours at room temperature in 50 ml of a medium containing 0.25 g magnesium sulfate, 0.5 g monopotassium phosphate, 10 g Difco peptone, and 20 g sucrose in 1 L of distilled water. The fungus growth was processed in a blender for 20–30 seconds, diluted in 450 ml of water, and poured on the surface of the soil around the plant.

The root-knot nematodes used were *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *M. hapla* Chitwood, 1949, *M. incognita* (Kofoid & White, 1919) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. incognita acrita* Chitwood, 1949, and *M. arenaria thamesi* Chitwood, 1952. Ectoparasitic nematodes used were *Helicotylenchus nannus* Steiner, 1945, and *Helicotylenchus buxophilus* (Golden, 1956) Perry, 1959.

Inoculum was obtained by culturing the nematodes on host plants growing in pots of steam-sterilized soil in the greenhouse. Each species or sub-species used in the different experiments was a sub-culture of one original population of that particular type of nematode. In most instances saprophytic nematodes also became established and no attempt was made to exclude or to eliminate them from the cultures. For inoculum, soil and roots containing the nematodes were mixed and about 25 cc was added to the appropriate pots at the time of planting. In the root-knot cultures gall formation varied and no attempt was made to determine the number of nematodes present. The minimum and maximum inoculum levels for *H. nannus*, determined by dilution counts, were about 200/pot in 1 experiment and about 32,500/pot in another. For *H. buxophilus* they were 230/pot and 2000/pot.

The experiments were of 3 categories: 1) short-term experiments in which 10 ml of the Fusarium inoculum was poured onto the soil around each potted carnation plant in half of the treatments 1 week after potting; 2) a medium-term experiment in which 10 ml of the Fusarium inoculum was poured onto the soil 2 months after potting; 3) a long-term experiment in which 20 ml of the Fusarium inoculum was poured onto the soil 5 months after potting. Plants used in the short- and medium-term experiments were potted in 2-in. clay pots, and all pots were buried in sterilized sand to within about ¼ in. of their tops in rows of 10 pots, each row being a replication of a treatment. Electric heating cables buried in the sand maintained the soil at about 85°F. In the long-term experiment 4-in. clay pots were used, each treatment consisted of a row of 5 pots, and soil temperature was controlled only by maintaining the greenhouse at a minimum night temperature of 60°F.

All tests were designed as randomized blocks with varying numbers of replications, and in all tests the following 6 standard treatments were applied as follows: 1) control; 2) adding *Fusarium* only; 3) wounding roots by stabbing through the soil at a point near the stem with a wooden pot label; 4) wounding roots and adding *Fusarium;* 5) adding nematodes only; and 6) adding both nematodes and *Fusarium*. The wounding in treatments 3 and 4 was done at the time of *Fusarium* inoculation.

Wilting or death of plants from any cause was recorded at weekly intervals for the short- and medium-term experiments and at monthly intervals for the

long-term experiments, beginning when the first plants wilted and continuing until the maximum effects of the *Fusarium* plus nematode infections were expressed in a treatment. At each reading the newly wilted or dead plants were counted. At the end of the experiment, wilt indices were calculated for each treatment by multiplying the number of wilted plants at the last reading by 1, the number at the next to the last reading by 2, the number at the third from the last reading by 3, etc. Therefore, a higher index occurs when more plants wilt or die earlier. There were 5 readings in the experiment shown in Table 1-A, 4 in the experiment shown in Table 1-B, etc. Thus the indices varied from experiment to experiment, and direct statistical comparisons cannot be made between experiments. All data were subjected to statistical analysis. (The calculations and much of the statistical analyses were by Mary K. Burk, Biological Aide, Nematology Section.) All references to significant difference mean statistically significant difference at the 1% level. Differences were compared by Duncan's multiple-range tests.

Experimental results.—The data from several representative experiments (Table 1) can be summarized, with the exceptions noted in the following paragraph,

Table 1.—Wilting index[a] for carnations inoculated with plant-parasitic nematodes (Meloidogyne spp. and Helicotylenchus spp.) at time of potting and with Fusarium oxysporum f. dianthi after 1 week, 2 months, or 5 months.

	No Fusarium		Fusarium		Synergistic Interaction
	Mean Indexes	Stat. Sig.[b]	Mean Indexes	Stat. Sig.[b]	
A (Fusarium after 1 week)					
Control	1.2	h	11.6	defg	
Root wound	0.4	h	17.6	de	−
H. buxophilus (2,000/pot)	1.6	h	14.0	def	−
H. nannus (32,500/pot)	13.2	def	18.0	d	−
M. arenaria	4.8	gh	35.0	bc	+
M. arenaria thamesi	5.4	gh	40.4	ab	+
M. hapla	0.0	h	37.0	bc	+
M. incognita	7.0	fgh	39.0	bc	+
M. incognita acrita	10.2	efg	47.2	a	+
M. javanica	2.2	h	32.6	c	+
B (Fusarium after 1 week)					
Control	4.0	ef	10.0	efg	
Root wound	4.0	ef	44.0	a	+
H. nannus (1,400/pot)	8.0	efg	18.0	bcde	−
M. arenaria	8.0	efg	21.0	bcd	+
M. arenaria thamesi	2.0	f	16.0	cdef	−
M. hapla	5.0	ef	18.0	bcde	−
M. incognita	6.0	ef	26.0	bc	+
M. incognita acrita	12.0	defg	27.0	b	+
M. javanica	6.0	ef	16.0	cdef	−
C (Fusarium after 1 week)					
Control	1.0	c	5.1	c	
Root wound	1.1	c	51.5	a	+
M. incognita	0.1	c	10.9	bc	−
M. incognita acrita	3.6	c	18.4	b	+
M. javanica	1.8	c	23.2	b	+
D (Fusarium after 1 week)					
Control	5.6	d	8.8	d	
Root wound	7.6	d	35.8	a	+
H. buxophilus (1,700/pot)	5.8	d	4.0	d	−
M. arenaria	6.0	d	21.4	bc	+
M. arenaria thamesi	6.6	d	15.4	cd	−
M. hapla	3.8	d	13.2	cd	−
M. incognita acrita	17.8	c	28.4	b	+
E (Fusarium after 2 months)					
Control	1.0	b	0.0	b	
Root wound	0.8	b	20.2	a	+
M. arenaria thamesi	0.8	b	20.0	a	+
M. hapla	0.0	b	2.0	b	−
F (Fusarium after 5 months)					
Control	0.8	d	1.1	cd	
Root wound	0.9	d	13.6	a	+
M. arenaria thamesi	4.8	b	12.6	a	+
M. incognita	11.3	a	13.9	a	−
M. incognita acrita	4.6	bc	10.4	a	+

[a] The wilting indexes were determined by scoring a wilted plant with a high value at the first observation and a lower value at each succeeding observation.

[b] Stat. Sig. means statistical significance at the 1% level. Mean indexes followed by one or more of the same letters are not significantly different (according to Duncan's Multiple Range Test).

as follows: There was a significant synergistic interaction between root-knot nematodes and *Fusarium*, i.e., the wilting index was higher in treatments with both *Fusarium* and root-knot nematodes than in treatments with either alone, and was also higher than the sum of their separate indices. Where the ectoparasitic nematodes *H. nannus* or *H. buxophilus* were used there was no interaction of nematodes and *Fusarium*. Root wounding or inoculation with nematodes without *Fusarium* did not increase the wilting index. *Fusarium* alone did not increase the wilting index, whereas *Fusarium* plus root wounding significantly increased the index. These results were the same in the 1-week, 2-month, and 5-month experiments. In these experiments there were no significant differences in the susceptibility of the different carnation cultivars to the *Fusarium* isolates.

There were 3 types of exceptions to these results: 1) *M. incognita acrita* (Table 1-A and D) and *H. nannus* (Table 1-A) caused an increase in the wilting index in short-term tests. *M. incognita acrita*, *M. incognita*, and *M. arenaria thamesi* all increased the wilting index in the long-term test (Table 1-F). 2) Although, in most experiments (as in Table 1-A) root-knot nematodes caused an increase in the wilting index in the presence of *Fusarium*, certain species in one or more experiments did not cause such an increase. The root-knot species that failed to cause this increase in the wilting index in some experiments were *M. javanica* (Table 1-B), *M. arenaria thamesi* (Table 1-B, 1-D), *M. hapla* (Table 1-B, 1-E), and *M. incognita* (Table 1-C). 3) In 1 test (Table 1-A), there was a significant increase in the wilting index of the controls with *Fusarium* over the controls without *Fusarium*.

Discussion.—The synergistic interaction of *Fusarium* and nematodes on carnations demonstrated in these experiments is similar to that of bacteria and nematodes on carnations (14) and of *Fusarium* and nematodes on several other crops (3, 4, 7, 8, 9, 10, 11).

In none of the experiments did the 2 ectoparasitic nematodes *H. nannus* and *H. buxophilus*, interact significantly with *Fusarium*. This is particularly interesting in view of the fact that the highest concentration of *H. nannus* inoculum alone did increase the wilting index (Table 1-A). The failure of injury by *H. nannus* to increase the wilting index significantly might be due to the differences in host pathogenic reactions to the different modes of feeding of endoparasitic and ectoparasitic nematodes and would seem to cast some doubt on the suggestions that the role of nematodes in *Fusarium*-nematode complexes of susceptible hosts is simply one of producing injury and a point of entry for the fungus (3, 4, 6, 7, 9). With resistant hosts it has been reported that the role of the nematode is more than one of providing a point of entry for the fungus (4, 6, 9, 15), although 2 later reports (1, 7) showed no effect of nematodes on the infection of resistant varieties.

These experiments show that both endoparasitic and ectoparasitic nematodes by themselves can cause significant injury to carnation plants. *M. incognita acrita* (Table 1-A, 1-D) and *H. nannus* (Table 1-A) increased the wilting index in short-term experiments. The effect apparently was caused by the initially high inoculum potential used since there was not time for appreciable reproduction and population build-up during the tests. The relative virulence of nematode species within short-term experiments cannot be accurately compared because the inoculum potentials were not standardized between species. All 3 root-knot forms used in the long-term experiment (Table 1-F) increased the wilting index in the absence of *Fusarium*. This increase was probably due to an increase in the nematode population during the 5-month experiment to a level that would cause injury.

Judging from the amount of galling on the roots of the host plants in the nematode cultures, the inoculum potential of a given species or subspecies varied from one experiment to another. In some experiments the cultures used were young and the inoculum level of all the forms was relatively low. Although the inoculum potentials of the root-knot nematodes were not quantitatively determined, it seems probable that low inoculum potentials were responsible in instances where the nematode-*Fusarium* interactions were not statistically significant.

The carnation cultivars used were susceptible to the Fusarium wilt fungus when root wounds were present. When roots were not wounded, however, all the carnations possessed a high degree of physical resistance to invasion or penetration. The Fusarium inoculum was applied 1 week, 2 months, or 5 months after carnations were potted in sterilized soil and grown without disturbing the root system in any way. When the root systems were undisturbed the wilting index did not significantly increase when Fusarium inoculum was used except in the experiment reported in Table 1-A. In that short-term experiment the *Fusarium* was added 1 week after the carnations were potted. One week is probably close to the minimum time required for healing of the root wounds made during potting the plants and for the barrier against invasion to become established. It appears likely that in this 1 test the wound healing process was delayed by some environmental condition.

These experiments demonstrate that root-knot nematodes in a carnation planting through interaction may cause a large increase in the amount and severity of Fusarium wilt. In addition to control measures against the Fusarium wilt organism, carnation plantings should be kept free of plant-parasitic nematodes. Nematodes may weaken the plants by feeding on the roots, as well as by increasing the seriousness of other diseases.—Plant Industry Station, Beltsville, Maryland.

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INFLUENCE OF TYLENCHULUS SEMIPENETRANS ON THE GROWTH AND CHEMICAL COMPOSITION OF SWEET ORANGE SEEDLINGS IN SOILS OF VARIOUS EXCHANGEABLE CATION RATIOS

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SUMMARY

The greatest reduction in growth of citrus seedlings from the presence of *Tylenchulus semipenetrans* was in soils maintained at levels of $CaCO_3$, Na, or K that were borderline or unfavorable for growth of uninfected plants. Leaf Cu was reduced by the nematodes in all the treatments, but was at deficient levels only where the soils were high in $CaCO_3$, Na, or K. Leaf Zn was also reduced on these same soils. Leaf Na was higher in the leaves of nematode-infected plants than uninfected plants grown in soils high in Na. The citrus nematode was reduced in numbers at pH values of 4.3 and 3.3, but not at pH values of 5.6–7.6.

Although the citrus nematode, *Tylenchulus semipenetrans* Cobb, occurs in all the citrus growing sections of the world, in most areas little specific information is available concerning the relative degree of growth retardation it causes. Citrus replants make apparently normal growth in the presence of the nematode in some California soils, and do very poorly in others. Controlled inoculation studies have indicated that the nematode may reduce growth about 10–50% (1). There is great need for information on why the nematodes damage trees more in one soil than another. It is possible that differences in soil chemical, physical, and biological properties other than the nematode population are of importance. With respect to soil chemical factors, Machmer (10) found more total nematodes and citrus nematodes around citrus roots subjected to continued higher, though tolerable salinity, than around citrus roots grown at lower salinity levels.

Godfrey and Hagan (3) found that a soil pH range of 4.0–8.5 had little influence on root-knot nematode activity in studies of the influence of soil hydrogen-ion concentration in Hawaiian soils. Kincaid and Gammon (6), in contrast, found the incidence of root-knot and root-lesion nematodes greater at pH 5.0 than at 6.2.

Little attention has been directed to the influence of the citrus nematode on nutrient absorption by the citrus plant. Several pertinent studies, however, have been undertaken with other nematodes. As early as 1903, Wilfarth and Wimmer (22) reported that nematode-infected beets from fertilization trials, had lower percentages of N, K, Na, Ca, and Mg than healthy beets. In 1943 Parris and Jehle (18) found that lima beans infected with root-knot were deficient in P despite the addition of sufficient P to the soil. In 1950 Tarjan (20) found the Mg, N, and K content of boxwood lower when infested with *Pratylenchus* sp. Na was higher in diseased leaves, stems, and roots. Oteifa (14, 15, 16, 17) followed with a series of investigations on the influence of K and N nutrition on the development of the root-knot nematode, and its effect on the mineral content of the host plant. He found that the length of time for egg production was inversely related to increasing K nutrition of the host. The K, Ca, Mg, N, and P contents were lower for infected bean plants than for healthy plants. He also found that ammonia ions decreased nematode injury as well as the number of females and egg masses produced on infected roots. Working with radioactive P,

Dropkin and King (2) concluded that tomato plants infected with root-knot and golden nematodes were not made unhealthy by deprivation of P by action of the parasite. Lownsbery (9) found the K content in leaves of walnut seedlings lower in plants exposed to large numbers of the root-lesion nematode than in uninfected plants. He found no effect of nematodes on Ca, Mg, Na, P, and Mn. Studying the pathogenicity of the same nematode, Sher (19) found the leaves of parasitized rose plants deficient in Fe, Cu, and K. Zn, Ca, Mg, and N concentrations were higher than in leaves of uninfected plants. There were no differences in P, Na, Mn, and Mo. Hunter (5), studying nutrient adsorption, found no indication of deficiency of P, K, N, Ca, Mg, Fe, and Cu content of root-knot nematode-infected tomato plants grown at ¼, ½, and 2 times Hoagland's solution in sand. The only significant difference Ohn (13) found when studying the nature of mineral deficiency symptoms of root-knot nematode-infected tomatoes, was a lower Na content of the roots. He found no differences in plants infested with the stubby-root nematode. Kirkpatrick et al (7) found that populations of the dagger Nematode and root-lesion nematode on sour cherries were correlated positively with leaf N and negatively with leaf K. They (8) also found that field applications of different levels of N and P did not significantly affect nematode numbers or kinds of nematodes. Vigor of trees was associated positively with levels of K application and negatively with numbers of nematodes.

In consideration of the deficiencies in available information, controlled greenhouse investigations were conducted to determine the influence of soil properties on growth and chemical composition of nematode- infected and uninfected citrus plants and the influence of soil properties on numbers of nematodes. This paper reports the results of a study in which the soil exchangeable Mg, K, and Na percentages were adjusted over the range normally found in southern California citrus orchard soils (4) and exchangeable H and Ca levels were varied from about 82% H or H plus Al (pH 3.3) to soil containing 0.5% excess lime.

Materials and methods.—Soils of various chemical properties were prepared as in studies of Martin and Bingham (12). The soils were leached in 3-gal. pots with about 2–3 L of 0.1*N* HCl per day until the pH of the leachate was 2.0, and then with similar quantities of distilled water until the leachate was free of chlorides. The soil was then dried, mixed, and analyzed for exchangeable cations. Exchangeable hydrogen or hydrogen and aluminum were neutralized with calculated amounts of the carbonates of Ca and Mg and the bicarbonates of Na and K to give the desired soil exchangeable cation ratios. The powdered chemicals were well mixed with the soil, and the soil was moistened and incubated for about 2 months. Special $CaCO_3$, low in alkali was used. After incubation the soils were again mixed and analyzed. Excess lime was added on the basis of the dry weight of the soil. About 4–6 months are required to process a 2- to 4-ton batch of soil in this manner.

Na, K, and Ca determinations were made by flame photometric procedures; Mg by precipitation as $MgNH_4PO_4$; Mn by the periodate oxidation; and Cu by dithazone, Fe by orthophenanthroline, Zn by Zincon, P by molybdic acid, B by carmine, and S by barium sulfate methods.

The soil treatments in Table 1 were superimposed on 2 different soils. The respective exchange capacities of soils in Experiment 1 and Experiment 2 were 11.8 and 15.3. The untreated mountain soil in Experiment 1 was used for comparison. The soil in Experiment 2 was high in organic matter and reacted abnormally in soil pH and base saturation relationships.

Before being planted, the soils were analyzed for inorganic nitrogen, and all pots in Experiment 1 were adjusted to 25 ppm with $CaNO_3 \cdot {}_4H_20$. After the plants were established, another 25 ppm nitrogen was added. Some of the soils in Experiment 2 contained 80 ppm inorganic nitrogen; therefore all pots in this series were adjusted to 80 ppm at planting time. In addition, all pots were fertilized with 1g of treble superphosphate, and those in Experiment 2 with 10 ppm Cu, 20 ppm each of Zn and Mn added as the sulfates, and 0.5 ppm B added as boric acid.

The sweet orange seedlings (*Citrus sinensis* Osbeck var. Homosassa) were inoculated in seedling flats 2 months after being planted. Citrus nematode larvae were collected from infected roots, washed 15 minutes in 1:1000 Cu SO_4 solution, and rinsed twice in dis-

Table 1.—The exchangeable cation analysis and pH of the soils in Experiments 1 and 2.

	Exchangeable cation percentages							pH after harvest	
Treatment	Ca	Mg	K	Na	Excess H	Excess $CaCO_3$	pH	Ck	Nema
Experiment 1:									
pH 4.3	22	5	2	1	70	0	4.3	4.3	4.3
pH 5.6	44	7	3	1	45	0	5.6	4.9	5.0
pH 6.3	66	9	4	1	20	0	6.3	6.2	6.2
pH 7.0	85	10	4	1	0	0	7.0	6.8	7.0
Excess $CaCO_3$	85	10	4	1	0	0.5	7.3	7.2	7.1
5% Na	81	10	4	5	0	0	7.2	7.0	7.1
9% Na	77	10	4	9	0	0	7.4	7.1	7.2
14% Na	72	10	4	14	0	0	7.6	7.3	7.2
9% K	80	10	9	1	0	0	7.1	6.9	6.9
18% K	71	10	18	1	0	0	7.3	7.1	7.0
26% K	63	10	26	1	0	0	7.5	7.1	7.2
50% Mg	45	50	4	1	0	0	7.2	7.1	7.1
Mountain soil	83	7	4	1	5	0	6.7	6.0	6.0
Experiment 2:									
pH 3.3	8	6	4	<1	82	0	3.3		3.4
pH 4.3	37	9	4	<1	50	0	4.3		4.1
pH 5.6	65	11	4	<1	20	0	5.6		4.9
pH 6.3	83	13	4	<1	0	0	6.3		6.0
Excess $CaCo_3$	79	17	4	<1	0	0.5	6.7		7.0
6% Na	78	12	4	6	0	0	6.6		6.2
11% Na	73	12	4	11	0	0	6.9		5.8
10% K	78	12	10	<1	0	0	6.6		6.1
20% K	68	12	20	<1	0	0	6.6		6.6
30% K	58	12	30	<1	0	0	6.6		6.3
32% Mg	64	32	4	<1	0	0	6.3		6.3
65% Mg	31	65	4	<1	0	0	6.9		6.5

tilled water. About 2–3 million larvae were added uniformly to each seedling flat. The seedlings were grown for another four weeks in the infested soil and then harvested and selected for uniformity. Healthy seedlings were harvested from uninfested flats. Life history studies (21) have shown that it takes about 4 weeks for larvae to develop into young swollen females attached to the roots. After that period uniform seedlings were transplanted to the various soil treatments without further addition of nematodes. Roots and soil were sampled for nematodes at the termination of the experiment. In Experiment 2 soil samples were taken every 6 weeks (one-generation cycle) to determine rate of increase in the nematode population.

Linear growth of the citrus seedlings was measured monthly, and total dry weights were obtained at harvest. Composite leaf samples (10 leaves per plant) were collected at harvest and prepared for mineral analysis.

Composite soil samples in Experiment 1 were analyzed for the dominant fungus flora, using Martin's Modified Medium (11).

Results.—The reduced growth of plants infected with citrus nematodes was highly significant in all the treatments of both experiments except at 9% K in Experiment 1 and pH 3.3 in Experiment 2 (Table 2). The only treatments that significantly influenced the uninfected plants were as expected: the low pH values, excesses of $CaCO_3$, and Na and K at the higher exchangeable percentages. The nematodes appeared to aggravate the situation under these conditions, increasing reduction in growth. Even at levels that had not affected the uninfected plants, growth reduction was increased. The nematodes had the greatest effect on the plants grown in soils containing an excess of $CaCO_3$, as shown in Fig. 1-A,B. Plants grown in acid to neutral soils were reduced 20–42% by the nematodes, compared to a 52–56% reduction in plants grown with an excess of $CaCO_3$. The K series in both experiments were variable but did indicate that the higher levels (18–30%) of K slowed growth more than the lower levels (4–9%). The absence of growth reduction at 9% K in the first test has not been explained. It appeared that a low Ca-Mg ratio reduced the effect of the nematodes on growth of the plants in Experiment 2. This point is being investigated further.

Important differences between nematode-infected and uninfected plants were found in leaf analysis. The level of Cu in leaves was less for nematode-infected than for uninfected plants in most treatments (Table 3). However, levels of deficiency in the nematode-infected plants occurred only in the alkaline soils as in Experiment 1 or in borderine soils such as Experiment 2, in which deficiencies occurred in all but the extremely acid soils. The greater reduction in growth of the nematode-infected plants in Experiment 2 was possibly due to severe copper deficiency. The copper available to nematode-infected plants was insufficient, even though copper was added to the soil at the beginning of the test. Cu deficiency symptoms developed on many of the nematode-infected plants after 2–3 months; whereas in the control soils deficiency symptoms were just becoming noticeable on some plants at time of harvest. This was also observed to a lesser extent in Experiment 1. Mild Cu, Mn, and Zn deficiency symptoms appeared on the nematode-infected plants 2 months after planting, particularly in the excess $CaCO_3$ and Na treatments. The growth of the nematode-infected plants was adversely affected throughout the experiments by these deficiencies. The results of the leaf analyses in Table 3 help substantiate the earlier observations of deficiency symptoms. The dry weights of the 10 leaf samples per nematode-infected plant were smaller than similar samples from uninfected plants, reflecting a size reduction from the nematodes (Fig. 1-A).

The level of Zn in the leaves followed much the same pattern as that of Cu, but was deficient only in the excess $CaCO_3$ and Na series. The level of Mn in nematode-infected plants was reduced in Experiment 1 but not in Experiment 2. The level of Na in the leaves of both uninfected and nematode-infected plants increased with increasing Na in the soil; however, nematode-infected plants took up nearly twice as much Na as uninfected plants. The level of Na in the leaves of the nematode-infected plants in Experiment 1 was enough to show some Na excess symptoms. Unpublished data substantiate the increase in Na content of leaves from nematode-infected citrus grown in soils containing

Table 2.—The average dry weights, % reduction, and number of citrus nematode larvae in Experiments 1 and 2.

Treatments	Av. dry wt. (g)/plant		% reduction due to nematode	Av. no. citrus larvae per 50 cc soil
	Check	Nema		
Experiment 1:				
pH 4.3	29	22	24	153
pH 5.6	38	31	20	764
pH 6.3	46	27	42	717
pH 7.0	39	25	36	514
Excess $CaCO_3$	24	11	56	421
5% Na	35	21	39	607
9% Na	38	22	42	698
14% Na	27	17	36	588
9% K	35	35	0	570
18% K	37	24	36	1020
26% K	32	22	32	928
50% Mg	37	31	17	936
Mountain soil	34	24	28	1424
LSD at 19:1	5			
LSD at 99:1	7			
Experiment 2:				
pH 3.3	5	5	0	1
pH 4.3	30	20	33	210
pH 5.6	46	39	16	810
pH 6.3	55	32	42	613
Excess $CaCO_3$	49	24	52	724
6% Na	46	22	53	508
11% Na	38	20	47	528
10% K	52	30	43	502
20% K	40	16	60	623
30% K	27	12	56	413
32% Mg	45	22	52	505
65% Mg	44	30	32	705
LSD at 19:1	6			
LSD at 99:1	8			

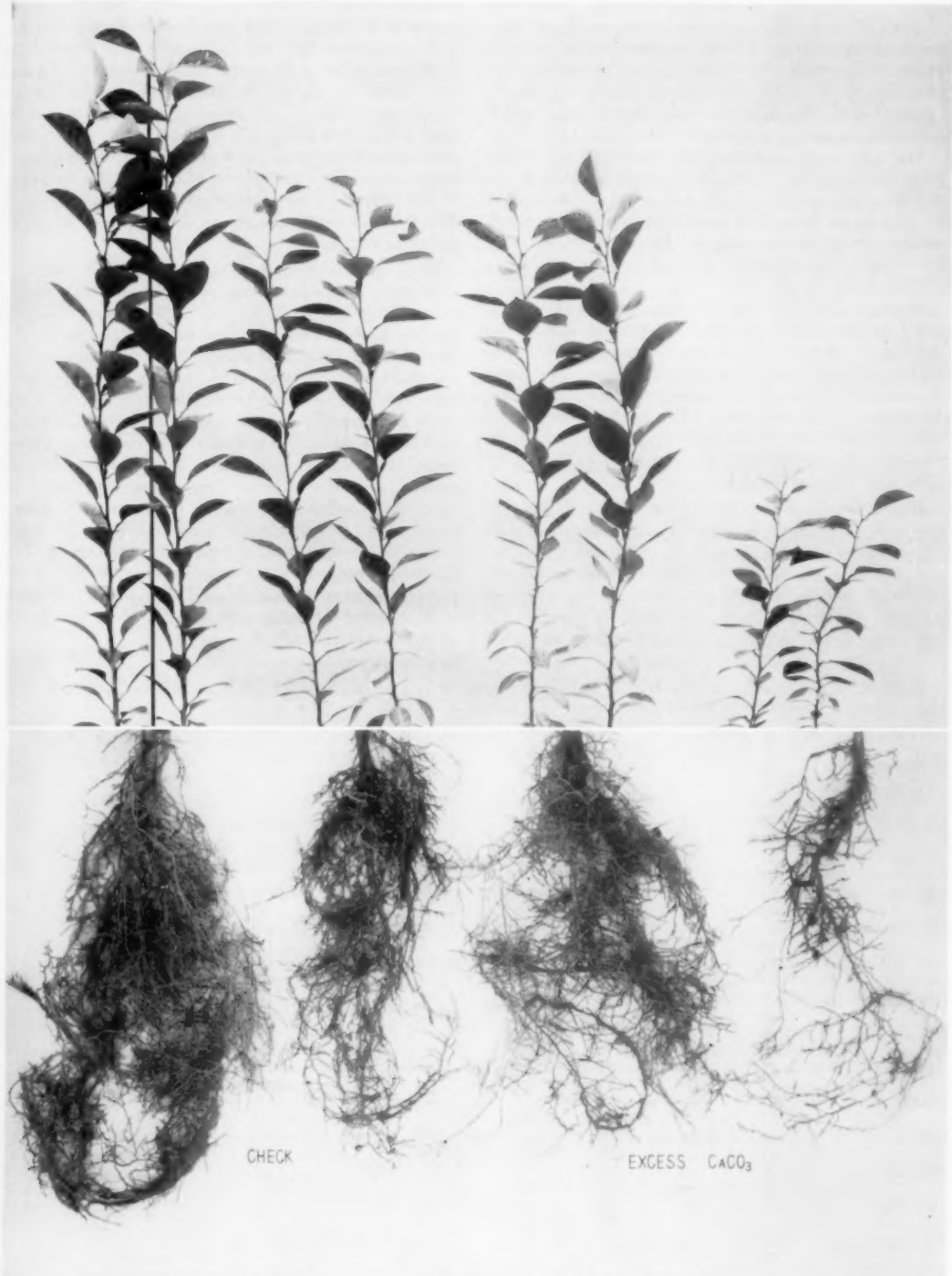

Fig. 1.—Above, top growth. Left to right: uninfected and nematode-infected plants grown at pH 7.0; and uninfected and nematode-infected plants grown at 0.5% excess $CaCO_3$. Below: matching roots.

relatively high levels of exchangeable Na. Treatments of high K and Mg produced correspondingly high levels in the leaves of both uninfected and infected plants. The leaves of plants grown at the highest level of K in both experiments contained excess amounts of K. The levels of P, Ca, Mg, K, Fe, S and B in the leaves were not affected by the nematodes.

The only soil treatment that had any significant effect on the citrus nematode population was pH. In the two experiments, soil pH values below 4.9 resulted in a reduced nematode population, giving a much slower rate of increase than at higher pH values (Fig. 2). Even so, the reduction in plant growth was highly significant at pH 4.3.

Analysis of composite soil samples for dominant fungi at the end of the first experiment showed no significant differences between treatments or between uninfected and nematode-infected plants. The most dominant fungi in all the treatments were *Penicillium fumiculosum*, *P. nigricans*, and *Trichoderma viride*. Other fungi were *Chaetomium* sp., *Fusarium solani*, *Penicillium chrisogemum*, *P. decumbems*, *P. islandicum*, and *P. ochro-chloron*.

Discussion.—Observations in the field indicate that the citrus nematode is capable of reproducing and living under a wide range of conditions from the desert to the coastal areas. This encompasses many soil types differing in both physical and chemical composition. Of the soil factors tested, only soils with a pH value below 4.9 decreased the citrus nematode populations over a period of 7 months. The nematodes were able to live at pH 3.3, though reproduction ceased. Such conditions are not ordinarily encountered in California, where most of the soils are neutral to alkaline. Only a few well-drained soils in San Diego County approach a pH value of 4.5. For the most part, soil acidity below pH 5 is not favorable for best citrus growth and may be in part the reason why fewer nematodes reduce the growth of citrus. The influence of pH may be more important in combination with different soil factors, such as soil texture, which is under investigation.

Although the nematode maintained itself under a wide range of conditions the reduction in plant growth due to the nematode varied. Effects were usually severest under conditions that were borderline or unfavorable for growth of citrus. Similar conditions occur quite often in many of the California citrus-growing areas. The most marked effect on the growth of the citrus was observed in soils containing excess $CaCO_3$ or high in exchangeable Na. Under these conditions, citrus made moderate growth when uninfected, and poor growth in the presence of citrus nematodes. Trees in these soils ordinarily showed symptoms of a minor element problem which was accentuated by the nematodes. This was particularly true with Cu and Zn. The nematodes caused an increased uptake of Na by the plants, and at times this reached toxic levels. Excesses of K affected nematode-infected plants in much the same manner as Na.

Table 3.—Concentration of minerals in leaves as determined by a composite leaf analysis of 10 leaves per plant. (The check plants are represented by the first line of each pair.)

Treatments	% Na	ppm Mn	ppm Zn	ppm Cu	Treatments	% Na	ppm Mn	ppm Zn	ppm Cu
Experiment 1:					*Experiment 2:*				
pH 4.3	.01	57	30	8.3	pH 3.3	<.03	65	30	6.4
	.01	47	32	4.4		.08	104	23	5.3
pH 5.6	.01	73	19	8.8	pH 4.3	<.03	30	21	4.8
	.01	59	27	4.0		<.03	44	21	3.8
pH 6.3	.01	46	22	4.0	pH 5.6	<.03	57	21	2.4[a]
	.01	55	24	3.3		<.03	76	19	2.3[a]
pH 7.0	.01	33	18	3.8	pH 6.3	<.03	38	21	1.8[a]
	.01	26	25	3.5		<.03	47	18	1.8[a]
Excess $CaCO_3$	.01	26	18	4.0	Excess $CaCO_3$	<.03	17	16	2.8[a]
	.01	20	15[a]	2.5[a]		<.03	26	14[a]	1.9[a]
5% Na	.01	48	19	4.3	6% Na	<.03	24	25	3.5
	.02	32	17	2.0[a]		.12	47	22	2.3[a]
9% Na	.03	35	20	4.0	11% Na	.10	58	23	2.3[a]
	.24[b]	24	14[a]	2.3[a]		.16	65	20	1.8[a]
14% Na	.11	46	22	4.5	10% K	<.03	54	24	3.3
	.26[b]	28	11[a]	2.5[a]		<.03	58	18	2.3[a]
9%K	.01	36	30	4.5	20% K	<.03	29	23	2.0[a]
	.01	23	15[a]	3.3		<.03	41	18	1.8[a]
18% K	.01	29	19	4.0	30% K	<.03	33	20	2.3[a]
	.01	26	18	2.6[a]		<.03	23	21	1.3[a]
26% K	.01	38	30	4.5	32% Mg	<.03	41	20	1.9[a]
	.01	39	23	2.7[a]		<.03	49	23	1.5[a]
50% Mg	.01	26	22	4.3	65% Mg	<.03	31	23	2.3[a]
	.01	16	22	3.3		<.03	33	22	2.3[a]
Mountain soil	.01	31	28	4.3					
	.01	31	24	3.4					

[a] Deficiency.
[b] Excess.

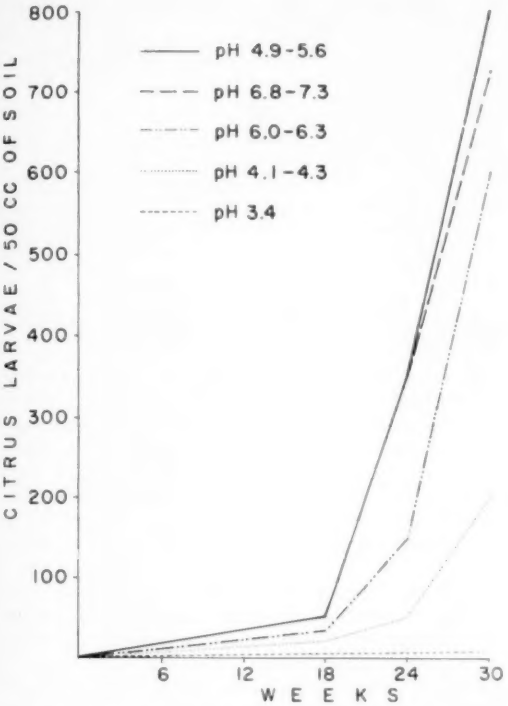

Fig. 2.—The increase in population of the citrus nematode in soils adjusted to different pH values.

When studying the influence of nematodes on the nutrition of host plants, considerable emphasis should be placed on knowing exactly the conditions the host plant is grown under. The mineral content of the citrus plant was little changed by the citrus nematode when grown in acid soils, but considerably affected in alkaline soils containing high amounts of Na or $CaCO_3$.—Departments of Plant Nematology and Soils and Plant Nutrition, University of California, Riverside.

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EFFECTS OF SELECTED CYTOSPORA ISOLATES FROM STONE FRUITS ON CERTAIN STONE FRUIT VARIETIES

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SUMMARY

Four *Cytospora* isolates from stone fruit trees were used in a study of strain virulence characteristics in plum, prune, and peach trees. It was concluded that such isolates range from saprophytic to vigorous wound pathogens, that they are not host-specific but are more damaging to some varieties than to others, and that infection progresses most rapidly when both nights and days are warm.

Introduction and review of literature.—Decline of plum and prune orchards has been recognized as a serious, multiple-factor problem in Idaho for at least 8 years. One of the most important of the causal factors has been the Cytospora canker disease complex.

Cytospora canker of plums and prunes is caused by a number of strains or forms of imperfect fungi, which, on occasion, appear to vary more within a group associated with a distinct species than between groups associated with related species.

The complexity of the Cytospora canker problem is reflected in the taxonomic confusion apparent in the literature. There is much synonymy (13, 16), inadequate description of environmental conditions, improperly substantiated conclusions; and often there is outright contradiction of apparently valid conclusions.

The *Cytospora* fungi are not new pathogens on fruit trees. There have been infrequent references to *Cytospora* diseases on stone fruits since Stewart et al. (14) first called attention to such a disease on peach trees (*Prunus persica* L.) in America, in 1900. Many controversial points have been investigated since then.

Aderhold's (1) reference, in 1903, to attack of frost-weakened trees was one of the first recognitions of the predispositional factors that have recently received close attention in Idaho.

By 1922, reports that the degree of parasitism depended on the isolate in question represented a major step forward in developing the concept of strains and strain variation. For example, of 3 strains of *Cytospora prunorum* Sacc. & Syd. isolated from cankered peach trees by Schwarz (12), 2 produced positive results when inoculated into healthy trees and 1 was entirely negative.

It was inevitable that consideration of contributing factors would lead into the additional complexities of seasonal extremes. Swiss workers (4) reported that damage to cherry trees by *Valsa leucostoma* was heavy 3 years after the severe winter of 1928–29. A similar problem of die-back in peach trees in Italy was attributed by Cristinzio (2) to *C. leucostoma*.

In 1935 Defago (3) conducted an extensive investigation of Valseae parasitic on declining stone fruit trees. He inoculated 39 species of *Prunus* with 1 form of *Leucostoma persoonii* (Nit.) Togashi and 2 forms of *L. cincta* (Fr.) Höehn., and concluded that nearly all were susceptible. After noting that wounds were necessary for infection, he concluded that the seriousness of the infection was governed both by strain virulence and host vigor. This condition for infection and these conclusions have been verified by observation and experiment in Idaho since 1951.

Working in Canada at about the same time that Defago was working in Switzerland, Willison (17) observed that cankers expanded continuously, or alternately expanded and partially healed, when peach trees were inoculated with *V. cincta* during the dormant season. This alternate expansion of canker margins and development of seemingly healthy callus at the margins has also been observed in Idaho plum orchards.

Willison (18) obtained maximum necrosis with inoculations made at leaf-fall. Summer inoculations were comparatively inocuous under certain conditions, another phenomenon occasionally observed in Idaho.

The variability and unpredictability of factors in the *Cytospora* disease complex on the stone fruit hosts are illustrated in practical terms by reports like that of Helton and Moisey (8). They noted that 5 *Cytospora* isolates from prune trees were severely pathogenic on seedlings of Lovell peach (*Prunus persica* L.), but produced no reaction on seedlings of sweet cherry or apple (*Malus pumila* Mill.). Such reports suggest a degree of host specificity not at all indicated by the results of Defago (3).

The investigations of orchard decline in Idaho (5, 6, 7) added several more fruit tree suscepts to the reported host range of isolates resembling *Cytospora leucostoma*.

These representative reports indicate that there has been confusion since the earliest investigations as to identification of *Cytospora*, or *Valsa*, isolates and pathogenicity of fungi presumed to be identical.

When the known stone fruit host range is considered, it is apparent that more information is needed regarding the relative virulence of different cultures from stone fruits on the same inoculated varieties under the same conditions, and regarding seasonal changes that occur in disease progress rates.

Materials and methods.—*Cytospora* isolates were taken from Italian prune, President plum and Bing sweet cherry, as indicated in Table 1.

Cankered stems were removed with pruning shears and surface-disinfested by swabbing with 95% ethyl alcohol in the laboratory. Pieces of tissue about 4 mm square were removed aseptically from astride the line separating dead and live tissues and transferred to

Table 1.—Sources of *Cytospora* isolates and disease conditions associated with them when collected.

Code	Time of collection	Host variety	Symptoms of the orchard tree
Cy-7	Nov., 1955	Italian prune	Dark, rusty-brown, depressed stem canker; foliage senescent, pale green to yellow terminal to canker.
Cy-8	Nov., 1955	Italian prune	Dark, rusty-brown, erumpent canker; tree defoliated and dormant.
Cy-9	Nov., 1955	President plum	Dark, cracked, gumming canker below much terminal dieback; tree defoliated and dormant.
Cy-11	Nov., 1955	Italian prune	Dark, rusty-brown, erumpent canker; tree defoliated and dormant.
Cy-12	Oct., 1955	Bing cherry	Slightly depressed and gumming canker with globular spore masses on pycnidial pustules.
Cy-16[a]	Feb., 1954	Italian prune	Dark, rusty-brown, erumpent and gumming canker; tending to girdle stems and form long subepidermal strands of necrotic tissue.

[a] Maintained on laboratory media between collection and use in this experiment.

culture plates containing quantities of standard laboratory potato-dextrose-peptone agar medium (11). The isolates were transferred to a malt-yeast extract agar medium (10) and examined for generic purity. Pure cultures of the isolates were maintained at 25°C until pycnidia developed in the culture plates. Single spores were isolated from the pycnidia to establish genetically pure cultures of each isolate.

In preparation of single-spore cultures, a pycnidium from each isolate was transferred aseptically to 10 ml of sterile distilled water in a test tube and agitated to disperse the pycnidiospores. One ml of the resulting spore suspension was transferred to a similar 10-ml water blank and agitated. This procedure was repeated twice more. From the final suspension, 1 ml was transferred to 20 ml of liquid synthetic medium (10) in a 125-ml Erlenmeyer flask. Flasks so prepared were incubated at 20°C until pinpoint colonies developed. One of these colonies was removed from each flask and placed on malt-yeast extract agar medium in culture plates. Using subsequently produced pycnidia from these plates, the entire procedure was repeated. The second group of cultures was presumed to be from single spores.

For the field-plot virulence study, the varieties were arranged randomly at a spacing of 6 ft. in each direction. Included in the plot were 36 Italian prune, 38 Stanley prune, 36 President plum, 12 J. H. Hale peach, 12 Rochester peach, and 12 Lovell peach trees. These were standard 2-year-old nursery trees procured from commercial nurseries and planted in April, 1956.

Inoculum for the plot study was prepared by aseptic mycelial transfers from stock cultures to malt-yeast extract agar plates, 20 ml per plate, and incubated 4 days at 25°C. Disks of medium 5 mm in diameter were removed from colony margins for inoculation of the trees. Sites on the trees selected for inoculation were swabbed with 95% ethyl alcohol. After the alcohol had evaporated, a cut was made averaging 4 mm deep, 14 mm wide, and 35 mm long, downward and inward along the axis of the stem at each selected site. This was done aseptically. Each cut thus exposed the cortical tissues and cambial region as well as a considerable area of xylem. A disk of inoculum was placed under each flap of host tissue immediately after the cut was made, with the mycelial surface toward the center of the stem. The flap was then bound back in place, over the inoculum disk, with nurseryman's rubber budding strips. After 2 weeks the rubber bindings were removed.

Three such inoculations were made on each tree. One was about 8 cm above the soil, 1 midway between the soil and the scaffold branches, and 1 on a succulent twig of current-season growth. The inoculations were made on August 4, 1956, and data recorded until April 12, 1957 (supplementary observations continuing through the summer).

An arbitrary numerical system was devised for recording reactions of the host plants to the *Cytospora* isolates, as follows: 0) no infection; 1) infected and cankered slightly; 2) gummosis at inoculation wound; 3) canker margin advancing slowly; 4) canker margin advancing rapidly; 5) stem girdled at the inoculation site; 6) all parts dead above the point of inoculation; 7) entire tree dead.

Results.—*Preliminary.*—A number of additional species and varieties have been found naturally infected with *Cytospora* spp. in Idaho, as follows: several apple and apricot varieties not identified; Bing sweet cherry (*P. avium* L.) and several other varieties; poplar (*Populus* spp.); Stanley prune (*P. domestica* L.); Montmorency sour cherry (*P. cerasus* L.); several peach varieties; western chokecherry (*P. demissa* (Nutt.) Walp.); several species of native and cultivated willow (*Salix babylonica* L.; *Salix* spp.); aspen (*Populus tremuloides* Michx.); and Russian olive (*Eleagnus hortensis* Bieb.).

Several isolation techniques were tried to determine the most efficient means of obtaining active cultures from diseased trees. These trials demonstrated that when tissue transplants were taken from astride the necrotic margin of a canker at its longitudinal extremes, 90% of the transplants could be expected to produce *Cytospora* in culture plates. If the transplants were taken from lateral margins of a canker, only about 10% developed *Cytospora*.

Four of the isolates used in the virulence study (Cy-7, Cy-9, Cy-11, and Cy-16) were identified as variants within *C. cincta* Fr., and the other 2 (Cy-8 and Cy-12) were considered to be strains of *C. leucostoma* Fr.

These identifications were based primarily on Willison's (17) descriptions of imperfect stages of certain *Valsa* fungi.

In orchards where the isolates were collected, numerous data were recorded on characteristics of symptom development and progress of the Cytospora diseases, particularly in plum and prune trees. These observations, made on preceding and following years as well as on the year of collection, are summarized as follows: Infection generally began in wounds made by freezing temperatures, orchard machinery, pruning tools, picker's shoes, ladders, and other harvest equipment; or almost any agent capable of breaking the bark. Young cankers were sunken or slightly swollen, but for the most part the cortex remained unbroken as the cankers expanded during the first season. Cracks were common in older cankers, and bark sloughed off in spots by the second season. If the margins of a canker were shaved at any season a line of demarcation was revealed beween necrotic and healthy tissues, generally a well defined line but occasionally quite diffuse. Canker margins were observed to expand in all directions, usually more rapidly upward along the longitudinal axis of the stem but sometimes rapidly also in a girdling or transverse direction. Discoloration and drooping (flagging) of foliage terminal to cankers was common prior to and during the girdling process. Such flags did not defoliate easily, and the leaves often hung on through most of the winter. Portions terminal to stem cankers died before or immediately after the stems had been girdled. During this time latent bud growth was often stimulated below the cankers. Such growth rarely survived longer than 1 season.

Exudation of gum from canker surfaces was common and often began soon after infection. This was governed to some extent by moisture conditions and fungus strain, but more profoundly by host variety. President trees gummed copiously, Stanley trees moderately, and Italian trees only slightly. Young peach trees and cherry trees of all ages also exuded abundant quantities of gum. The gum remained in globular masses on the canker surfaces or, when moisture was plentiful in the environment, oozed down the stems in streaks reminiscent of the bleeding of fire-blight cankers on pear trees. Except for temporary halting during unfavorable seasons, or artificial interruption of their progress, canker margins continued to expand until the trees were dead.

Pycnidial pimples were formed in canker surfaces behind the advancing margin; closeness to the margin, time required for formation, and number formed per unit area apparently determined by strain of fungus, host variety, and environmental factors such as temperature. The pycnidial pustules, or pimples, developed from stromatic masses in and below the periderm. At sporulation the surface over the ostiolar portion of the pustule ruptured and spore masses were extruded —in long hairlike filaments during dry conditions and in globular masses during wet weather. The spore masses were generally amber-colored during the first season, and had disappeared by the second season, whitish spots marking their former locations.

Areas in infested orchards where Cytospora diseases were most damaging were generally those where predisposing conditions were most prevalent. Trees in low spots of excessive moisture and winter cold were often damaged the most. However, areas of severe damage were often found in a random arrangement not associated with such contributing factors.

Virulence study.—Four plot trees each of the President and Italian varieties and 5 of the Stanley variety were inoculated with each isolate. Cy-8, Cy-9, Cy-11, and Cy-16 were inoculated into 2 trees each of J. H. Hale and Rochester peach, and 1 of Lovell peach. Cy-12 was inoculated into 1 Hale, 2 Rochester, and 2 Lovell trees. Cy-7 was inoculated into 2 Rochester and 2 Lovell trees. Check trees receiving aseptic agar disks were 4 President, 4 Italian, 5 Stanley, 1 Hale, and 2 Lovell. Fig. 1 summarizes the data.

In general the cankers formed by isolates Cy-9, Cy-11, and Cy-16 expanded most rapidly during warm weather (60–81°F), and slowly during cool weather (35–60°F). There was no appreciable advance of margins during cold periods (35°F and below). Margin advance was most rapid during warm days and nights, averaging 63°F. Advance was slow during warm days and cool nights, averaging 44°F. No advance was observed during cool days and cold nights, averaging 32°F. As to averages for seasonal periods, margins advanced most rapidly from the time of inoculation, on August 4, to early September. In some cases there was a slow advance from mid-September to late October. No measurable advance occurred from late October to early February. The temperature ranges of these 3 periods are indicated above. During early February to mid-April, a slow advance resumed in some cases, and in the following summer it became rapid again.

The margins of cankers formed by the less virulent isolates Cy-8 and Cy-12 advanced only slowly during the period of greatest fungus activity, i.e., from August to early September, and advanced not at all during the winter (Fig. 1). Slight advance was observed again after mid-February.

During September, most of the inoculated trees developed callus ridges at canker margins, which suggested that *Cytospora* fungi were being walled off and progress of the disease halted. This duplicated a condition frequently observed in President plum orchards in commercial areas. Nevertheless, as also observed in the President orchards, the cankers on the experimental trees began to expand beyond the callus ridges during the 2-month period following mid-February. Little or no callus developed on Italian trees; in other varieties the amount of callus appeared to be governed largely by the vitality of individual trees.

As indicated in Fig. 1, Cy-7 was slightly infectious on peach trees but completely innocuous on plum and prune trees.

Exudation of gum from infected stems appeared to be a function of both isolate and host variety (Table 2).

All check trees healed normally.

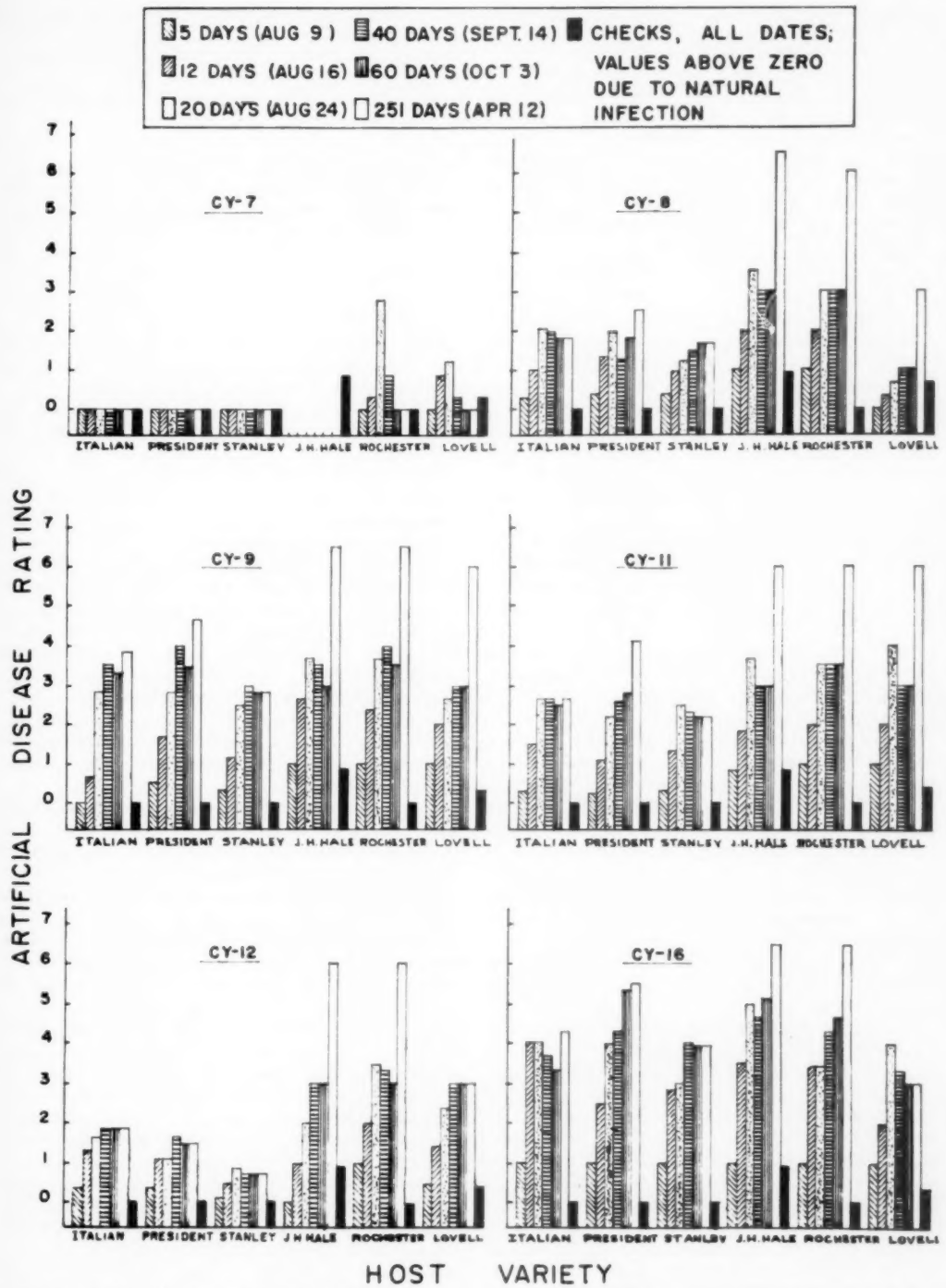

Fig. 1.—Pathogenicity of 6 *Cytospora* isolates on 2-year-old trees of 3 plum and 3 peach varieties in a field plot inoculated August 4, 1956. Each isolate was inoculated into 4 trees of each plum variety and 2 of each peach variety. Each tree was inoculated near the soil, midway up the trunk, and on a current-season branch. Inoculation sites were rated from 0 (no infection) to 7 (tree death). Each bar value represents the average of the 3 inoculation sites on each tree and the number of trees per variety.

Table 2.—Production of gum in association with artificially induced Cytospora diseases on 1- and 2-year-old stone fruit trees in a field plot.

Isolate	Stone fruit variety						
	Italian	Stanley	President	Hale	Rochester	Lovell	Checks
Cy-7	0[a]	0[a]	0[a]	—	0[b]	0[b]	0[a]
Cy-8	none	slight	moderate	moderate	moderate	none	0[a]
Cy-9	slight	moderate	copious	none	copious	moderate	0[a]
Cy-11	none	moderate	moderate	copious	moderate	none	0[a]
Cy-12	none	none	none	none	none	none	0[a]
Cy-16	none	moderate	copious	copious[c]	copious	moderate	0[a]

[a] No cankers formed; no infection.
[b] Slight gummosis at inoculation wounds.
[c] Exudation from stems between cankers as well as from canker surfaces.

Reisolations.—On completion of the virulence study, reisolations of the causal fungi were made and the reisolates compared with the original cultures. The comparisons were made both on potato-dextrose-peptone agar and a synthetic medium described by Lilly and Barnett (10) to which agar had been added and which contained D-levulose as a carbon source. The first was used for comparison of colony characteristics and the second for comparison of sporulation characteristics.

After the reisolates were purified, 2 plates of each medium were inoculated with each original isolate and each reisolate. Isolate Cy-7 was omitted because attempts to reisolate it failed. The plates were incubated 11 days at 25°C.

The colonies of original isolate Cy-8 and reisolate Cy-8 were identical, i.e., brownish with irregular borders and cottony surface mycelia, with beaked pycnidia scattered over the surfaces.

The colonies of original and reisolate Cy-9 were also alike, i.e., subsurface olivaceous mycelia in the centers of colonies with very irregular margins and sparse, felt-like pycnidia scattered over the surfaces.

The colonies of original isolate Cy-11 were cottony gray with regular margins, black to olivaceous subsurface mycelia in the centers, and felt-like pycnidia scattered over the surfaces. The reisolate colonies had the same characteristics except that the subsurface mycelia were black.

The colonies of original and reisolate Cy-12 were like those of isolate Cy-8 except that they were slightly darker brown.

The colonies of original isolate Cy-16 were prominently zonate and cottony white, often with a pink tinge. The zonations were formed of alternating bands of dark and light olivaceous subsurface mycelia. Felt-like pycnidia were scattered over the colony surfaces. The reisolate colonies were essentially the same except that the subsurface mycelia were darkly olivaceous to black. Naked conidiophores (such as reported by Hildebrand (9)) of original isolate Cy-9, Cy-11, and Cy-16 were formed by dichotomous branching of hyphal tips. The resulting conidiophore masses ranged from 10 to 1,000 μ in diameter. Original isolates of Cy-8 and Cy-12 developed spiny mycelial filaments that then branched and formed similar masses of conidiophores. All reisolates performed as did the originals.

Both the originals and reisolates of Cy-9, Cy-11, and Cy-16 produced hyaline spores of allantoid shape and of variable length averaging 6.6 μ. The pycnidiospores were the same size, shape, and color as the conidiospores, the only observed difference being that the former were produced in pycnidia and the latter were not.

The pycnidiospores and conidiospores of original isolates Cy-8 and Cy-12 appeared to be alike: hyaline, allantoid, and of varied length, averaging 6.7 μ. The reisolates were the same.

Discussion.—The isolates of *Cytospora* used in this study were considerably more stable in culture but less host-selective than isolates from the same commercial areas described by Helton and Moisey in 1955 (8). Evidence of this stability was reflected in the fact that reisolations of the causal fungi from the field-plot trees produced sporulating cultures like the originals even though cultural mutations, evidenced by development of triangular segments in colonies were rather common during laboratory manipulation of the isolates.

Not all the isolates were pathogenic, as demonstrated by the generally saprophytic behavior of Cy-7, which is in agreement with Wormald's conclusions (19) regarding *Cytospora* isolates from cherry. This also agrees with Schwarz's conclusions regarding physiologic strains of the fungi (12).

The virulence study demonstrated that the degree of pathogenicity in a given instance is dependent on both fungus strain and host variety, which parallels the conditions noted by Defago (3).

The effects of strain differences, within each species group, in the production of disease were noted in several instances. Such differences were even more prominent between the species groups, as was demonstrated by slow margin advances in most varieties by Cy-8 and Cy-12, and inconsistently rapid advances by other isolates.

These points do not necessarily verify the supposition entertained at the start of the study, that *Cytospora* isolates from plum and prune trees might possess demonstrable host-specificity characteristics, but they do reveal that different isolates of the fungi do not parasitize different varieties at equal rates. Neither does a single isolate parasitize a given variety at the same rates under different seasonal conditions. Rates

of disease development during summer weather were most rapid when the nights also were warm, which is in agreement with laboratory and pathogenicity results reported by Treshow (14) for *C. rubescens*. This is an environmental factor that could account for differences in orchard damage between areas of high vs. low nocturnal temperatures.

Cytospora canker diseases were exceedingly severe following the extreme winter injury sustained in stone fruit orchards in Idaho in November of 1955. This parallels the report of Faes, Staehelin, and Bovey from Switzerland in 1932 (4).

Exudation of gum from infected stems has been a commonly encountered characteristic in commercial orchards since 1951, and especially since 1955. Both Table 2 and Fig. 1 (cf. definition of arbitrary system, materials and methods) illustrate that stem gummosis tendencies are related both to fungus strain and host variety. This is reflected in the fact that Cy-9 and Cy-16 were comparably vigorous in their abilities to expand newly formed cankers and to produce gum from those cankers, and in the fact that the Cy-12 isolate from Bing cherry was moderately pathogenic on test trees (Fig. 1) but produced no gum at all on the canker surfaces (Table 2). The varietal effect is also demonstrated by the tendency of the President plum to gum extensively, the Stanley moderately, and the Italian almost not at all (Table 2).

The means by which *Cytospora* fungi kill host tissues are not well understood and should be investigated further. During hot weather the portions terminal to cankers suddenly die as though from an interruption in the water supply. From peach and grape investigations Willison suggested that a fungus toxin might be responsible. Helton and Moisey (8) supported this idea. Some foundation for it also can be found in the present study, as illustrated by gummosis of Hale stems in the areas between cankers caused by Cy-16. If a toxin is responsible it does not move downward in infected stems at an appreciable rate, since portions below cankers generally remain healthy much longer than those above, and if infected portions are cut off below the canker margins the disease is halted. However, since the fungi can be isolated from deep within the xylem of infected stems, it is not likely that toxins are the only factors involved.

The plot used in the virulence study was situated astride a poorly drained area, and the trees were randomly arranged with regard to both variety and isolate. There appeared to be no correlation between the poorly drained area and isolate virulence. Since this correlation seems to exist in commercial orchards, more time will be required to demonstrate it experimentally.

Conclusions.—1) Identification of *Cytospora cincta* and *C. leucostoma* as the primary causal species in plum and prune orchards in Idaho, as exemplified by the isolates used, can be made on presumptive bases that disregard the failure of all attempts to induce *Valsa* stages from these species. 2) Isolates obtained from seriously diseased sections of the stems of stone fruit trees are not necessarily vigorous pathogens, or even pathogenic at all. 3) Although epiphytotic conditions have developed in Idaho only after periods of severe low-temperature injury, simple mechanical wounding of healthy trees provides adequate entry courts for *Cytospora* fungi. 4) Gummosis of infected stems, is governed as much by host variety as by fungus strain. 5) *Cytospora* isolates from stone fruit trees are not entirely host-specific, i.e., pathogenicity on young trees is governed to some extent by host variety but predominantly by virulence characteristics of individual fungus strains. 6) Rates of disease development in summer-inoculated trees are rapid during the first 3 weeks of infection but level off during the second 3-week period. 7) Disease development proceeds rapidly during warm weather but most rapidly when the nights also are warm.—Department of Plant Pathology, University of Idaho Agricultural Experiment Station, Moscow.

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STRAINS OF PSEUDOMONAS SOLANACEARUM DIFFERING IN PATHOGENICITY TO TOBACCO AND PEANUT

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SUMMARY

Isolates of *Pseudomonas solanacearum* obtained from tobacco, peanut, tomato, eggplant, and Irish potato from North Carolina, South Carolina, Georgia, and Florida were highly pathogenic on Irish potato, tomato, and eggplant; no significant differences between isolates were demonstrated in stem inoculations of these hosts. However, there were marked differences among isolates from tomato in pathogenicity on tobacco and peanut. In particular, tomato isolates from Georgia were less pathogenic on tobacco than tomato isolates from North Carolina. Certain isolates that were avirulent on tobacco were highly virulent on peanut, but the reverse was true for other isolates. Results indicate the presence in the Southeastern United States of strains of *P. solanacearum* that differ in pathogenicity to hosts such as tobacco and peanuts. Isolates from Israel and Cyprus, originally from Irish potatoes, were highly pathogenic on tomato and eggplant but low in virulence on tobacco and peanut, both in stem and root inoculations.

Evidence for the existence of pathogenic strains of *Pseudomonas solanacearum* E. F. Sm., causal agent of southern bacterial wilt or Granville wilt, has been mainly observations of natural occurrence in different parts of the world (11). No experimental evidence has been presented that such strains exist in the United States. In extensive cross-inoculation experiments, Stanford and Wolf (22) detected no strain differences among isolates of the wilt bacterium from many different hosts in North Carolina. Although the rare appearance of bacterial wilt on peanuts in North Carolina might be considered evidence of a strain weakly pathogenic on that crop, Fulton and Winston (4, 5) proved that isolates from peanuts in North Carolina were pathogenic to tobacco, and vice versa.

In Georgia, bacterial wilt is infrequent on tobacco, but is relatively common on tomato in tobacco-growing counties and to a lesser extent on peanut (6, 16). In 1954 the junior author observed a tomato transplant field in the vicinity of Tifton, Georgia, in which 90% of the seedlings in a three-acre field were killed by *P. solanacearum*. This field was planted with the susceptible Hicks tobacco variety the following year but not a single plant showed symptoms of bacterial wilt. No cross-inoculation studies with isolates from tobacco and tomato in Georgia have been reported, however.

In other parts of the world, including Southern Rhodesia (9, 10), the Union of South Africa (17, 23, 24), Australia (7, 8), Portugal (2), and Brazil (3), strains have been reported that were pathogenic on Irish potato or other solanaceous hosts but would not attack tobacco. Okabe and Goto (18, 19) indicated that a number of pathogenic strains were present in Japan.

More recently, isolates from banana, *Heliconia*, and solanaceous hosts in the Coto region of Costa Rica have been found that differ in virulence in cross-inoculation studies (1, 20, 21). Isolates from solanaceous hosts were avirulent on banana and isolates from banana were weakly virulent on solanaceous hosts.

The rapid loss of virulence of *P. solanacearum* maintained in culture by routine methods, is well known (11). Thus, any evaluation of possibly distinct strains in the Southeastern United States requires that all isolates tested should be cultures of the virulent colony type, so that levels of virulence on susceptible plants can be compared properly. With the development of techniques for recognition of colonies of the virulent type (12) and of methods for maintaining viability and pathogenicity in storage (13, 14), work was initiated in 1954 to determine whether pathogenic strains of *P. solanacearum* are present in the Southeastern United States. A preliminary report has been presented (15). Comparisons were also made with isolates of *P. solanacearum* from Israel and Cyprus.

Materials and methods.—Isolates were obtained from the following economic hosts: Irish potato, tobacco, peanut, tomato, and eggplant. These isolates were collected in North Carolina, South Carolina, Georgia, and Florida; isolates from Israel and Cyprus were respectively obtained from Dr. Zafrira Volcani and Dr. W. J. Dowson. Table 1 shows specific data on the isolates, including the hosts from which obtained. Stock suspensions were prepared by placing 3–5 loopfuls of bacteria from isolated colonies into capped test tubes containing 5 ml of sterile distilled water. These suspensions were stored at a temperature of 22°C. In this manner, cultures could be maintained relatively free of avirulent mutant types for 18–24 months.

Test plants used were: Bottom Special and Dixie Bright 101 tobacco (*Nicotiana tabacum* L.); Rutgers and N.C. breeding line 114 tomato (*Lycopersicon esculentum* Mill.); N.C. 2 peanut (*Arachis hypogaea* L.); Irish Cobbler potato (*Solanum tuberosum* L.); and Black Beauty eggplant (*Solanum melongena* L.). Except for resistant Dixie Bright 101 and N.C. 114, all host plants were considered highly susceptible to bacterial wilt. The plants were grown from seed in flats, transplanted to 6-in. pots containing a mixture of field soil and sand, and maintained in a succulent growing condition.

Bacterial suspensions used in inoculations were pre-

pared by removing 1–3 loops of bacterial slime from the surface of 48- to 72-hour-old cultures streaked on tetrazolium chloride (TZC) medium (12), and placing the loopfuls in 5 ml of distilled H_2O. The suspensions were adjusted by turbidity measurements with a Fisher electrophotometer to contain a minimum of about 10^8 cells per ml. To insure that all suspensions were prepared from the virulent fluidal colony type of *P. solanacearum*, all cultures on the TZC medium were examined before preparation of the inoculum (12).

Stems were inoculated by placing a drop of bacterial suspension on the leaf axil of the third expanded leaf below the stem tip (24) and pushing a sharp arrowhead needle through the droplet into the vascular tissue of the stem to permit rapid uptake of the inoculum. Roots were inoculated by cutting roots to a depth of 2 in. along one side and pouring 25 ml of the bacterial suspension over severed roots in the cut area. Eight to 10 plants of each host were inoculated with each isolate. Inoculations were repeated 2 or more times on each of the hosts.

Disease readings were taken at 7-day intervals following the method outlined by Winstead and Kelman (25). Following inoculation, greenhouse temperatures were maintained at about 22°C at night and 30–32°C in the day.

Experimental results.—All isolates were classified as to virulence on the basis of disease index ratings. Table 1 summarizes the results of tests with selected isolates.

Of the plants used, Irish potato was the most susceptible. In one test, 10 days after inoculation with a representative group of isolates, all inoculated plants had wilted completely. Under greenhouse conditions optimal for disease development with *P. solanacearum*, Irish potato plants were highly susceptible to infection and isolates could not be differentiated on this host. Similarly, differences between isolates were not evident in the inoculation tests with eggplant and the wilt-resistant tomato (N.C. 114).

There was considerable variation from test to test in disease severity for all isolates, although isolates generally maintained the same rank order on a given host in successive tests. Of the isolates tested, 60-R-2, originally from tomato and then passed twice through Dixie Bright 101 tobacco, was the most uniformly highly pathogenic on the hosts tested.

There were marked differences among isolates from Georgia in relative pathogenicity to tobacco and peanut. In general, the tomato isolates from Georgia were weakly pathogenic on tobacco (See Fig. 1). One tomato isolate (77 from North Carolina) was only moderately virulent on tobacco.

These differences in virulence of isolates were more evident on the resistant Dixie Bright 101 than on susceptible Bottom Special. On Dixie Bright 101, only 2 of 16 isolates from tomato in Georgia were highly virulent; the others were moderate to low in virulence. All 3 North Carolina isolates from tobacco were highly virulent on this host. Certain isolates that were avirulent on tobacco were highly virulent on peanut, but the reverse was true for other isolates.

Table 1.—Relative virulence of isolates of *Pseudomonas solanacearum* obtained from different economic hosts and geographic areas.

Isolate No.	Host	Area	Relative virulence[a] to different hosts[b] IP	Eg	Tm	Tb	Pe
3	Potato	Pamlico Co., N. C.	H	H	H	H	–
25	Tomato	Tift Co., Ga.	–	–	H	L	H
31	Peanut	Northampton Co., N. C.	H	–	H	H	H
45	Tobacco	Wake Co., N. C.	H	H	H	H	L
46	Tobacco	Granville Co., N. C.	H	H	H	H	M
56	Potato	Israel	–	–	H	L	L
57	Potato	Israel	–	–	H	L	L
60-R-2	Tomato	Wake Co., N. C.	H	H	H	H	H
61	Tomato	Berrien Co., Ga.	H	H	H	L	M
62	Tomato	Pierce Co., Ga.	H	H	H	M	H
63	Tomato	Evans Co., Ga.	H	H	H	M	L
64	Tomato	Worth Co., Ga.	H	H	H	M	L
65	Tomato	Berrien Co., Ga.	H	H	H	M	H
66	Tomato	Berrien Co., Ga.	H	H	H	L	M
67	Tomato	Bacon Co., Ga.	H	H	H	M	M
68	Tomato	Coffee Co., Ga.	H	H	H	M	H
69	Tomato	Cooke Co., Ga.	H	H	H	L	M
70	Tomato	Irwin Co., Ga.	H	H	H	L	M
71	Tomato	Berrien Co., Ga.	H	H	H	L	M
72	Tomato	Colquette Co., Ga.	H	H	H	L	H
73	Tomato	Colquette Co., Ga.	H	H	H	M	M
74	Tomato	Worth Co., Ga.	H	H	H	M	H
75	Tomato	Turner Co., Ga.	H	H	H	M	L
76	Tomato	Cooke Co., Ga.	H	H	H	M	L
77	Tomato	Northampton Co., N. C.	H	H	H	M	M
82	Eggplant	New Hanover Co., N. C.	–	–	H	M	–
101	Eggplant	Edisto Station, S. C.	–	–	H	H	–
105	Tobacco	Quincy, Fla.	–	–	H	H	–
109	Tobacco	Jeff Davis Co., Ga.	–	–	H	H	–
112	Eggplant	Faison, N. C.	–	H	H	M	–
118	Potato	Cyprus	–	–	H	L	L

[a] Disease indices based on ratings of disease severity of 0–5 converted to percentage. Ratings made 21 days after inoculation. H = high, 65–100%; M = moderate, 40–65%; L = low, below 40%.

[b] Host designations: IP = Irish potato; Eg = eggplant; Tm = tomato; Tb = tobacco; Pe = peanut.

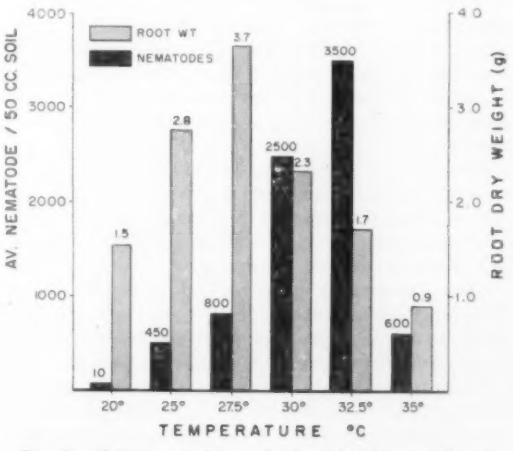

Fig. 1.—Relative virulence of selected isolates of *Pseudomonas solanacearum* from North Carolina and Georgia. Isolate No. 46 was from tobacco; all others from tomato. Disease indices based on ratings 21 days after stem-inoculation of resistant Dixie Bright 101 tobacco plants.

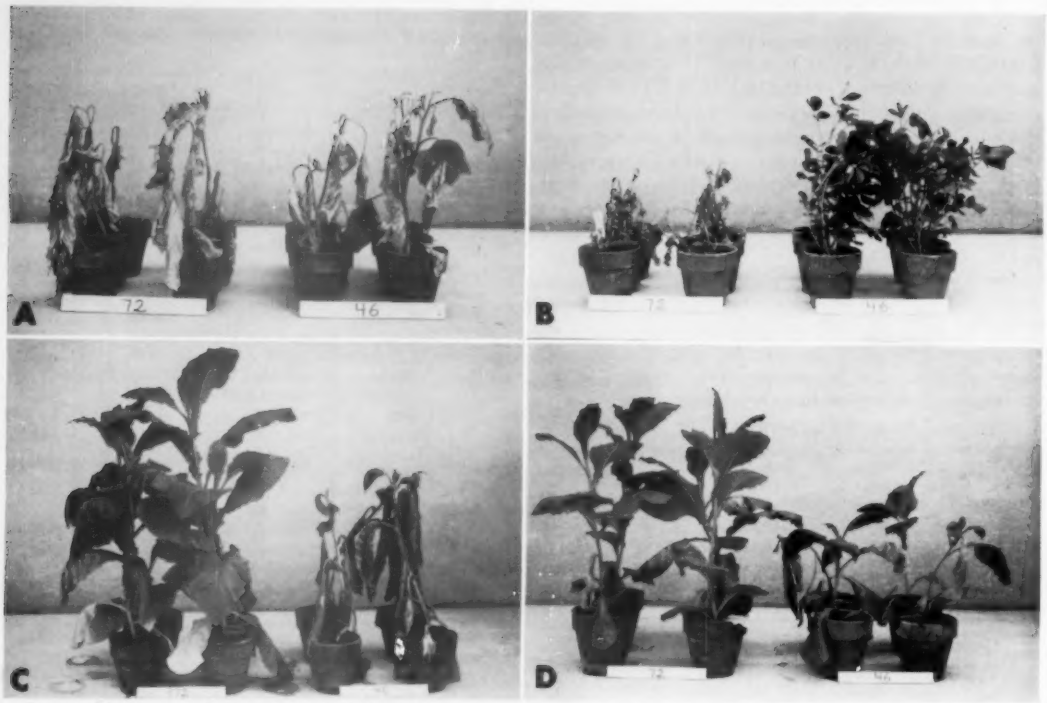

Fig. 2.—Symptoms of bacterial wilt 14 days after stem inoculation of plants with isolate 72 (Georgia, tomato) left, and 46 (North Carolina, tobacco) right. **A)** Black Beauty eggplants; **B)** N.C. 2 peanuts; **C)** Bottom Special tobacco; **D)** Dixie Bright 101 tobacco.

Furthermore, certain isolates were equally pathogenic on both hosts.

Fig. 2 shows eggplant, tobacco, and peanut plants inoculated with 2 isolates, one from tobacco in North Carolina (isolate 46) and one from tomato in Georgia (isolate 72). Isolate 72 was highly pathogenic on eggplant and peanut but weakly pathogenic on tobacco; in contrast, 46 was highly pathogenic on eggplant and tobacco but weakly pathogenic on peanut.

In 2 tests, the isolate from Irish potato from Cyprus was not pathogenic on tobacco. The isolates from Israel in both stem and root inoculations were pathogenic on tomato but low in virulence on tobacco and peanut.

General wilt severity was significantly lower from root inoculations than from stem inoculations. Ranking of isolates, however, was generally similar.

Discussion.—This investigation confirms field observations in Georgia of strains of *P. solanacearum* of low virulence on tobacco. Evidence that isolates from solanaceous hosts in Georgia and North Carolina differ in virulence in cross-inoculations supports the findings of Okabe and Goto (18, 19) in Japan.

Strains capable of attacking tomato, as well as strains capable of attacking both tomato and tobacco, are apparently co-extensive in wide areas. It appears, however, that bacterial wilt on tomato in certain areas does not necessarily indicate a hazard for tobacco or for peanuts in these areas. In general, all isolates obtained in the Southeastern United States can be considered to be highly pathogenic to potato, eggplant, and tomato, since they were able to affect these hosts under greenhouse inoculation conditions. Typing the number of different strains in the Southeastern United States will require much more extensive testing of isolates, using tobacco and peanut as the differential hosts.

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COMPLEMENTARY ISOLATES OF PHYTOPHTHORA PALMIVORA FROM CACAO AND RUBBER, AND THEIR TAXONOMY

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SUMMARY

In an examination of over 200 isolates of *Phytophthora palmivora* from cacao and rubber from 22 different countries, complementary strains of the pathogen were found on both hosts. In general, oospores were readily produced in mixed cultures of isolates from rubber and cacao. Justification for raising isolates from the two hosts to varietal level within the species is considered inadequate.

Introduction.—Orellana (4) recently described experiments in which 6 isolates of *Phytophthora palmivora* (Butl.) Butl., a ubiquitous pathogen of *Theobroma cacao* L. and *Hevea brasiliensis* Muell.-Arg., were used to illustrate differences between isolates attacking the two hosts. He suggested that incompatibility and specificity of isolates from the two hosts warranted transfer of the isolates to varietal level, i.e., *P. palmivora* var. *theobromae* and *P. palmivora* var. *heveae*. A recent survey of the disease (7, 8) at the West African Cocoa Research Institute, Ghana, has, however, failed to confirm the findings of Orellana or to justify the suggested taxonomic changes. The results of comparative tests on *P. palmivora* from 22 different countries are reported in this paper.

Materials and methods.—Isolates of *P. palmivora* were obtained from cacao in Angola, Belgian Congo, British Guiana, Cameroun, Ceylon, Dominica, Fernando Po, Gabon, Ghana, Ivory Coast, Jamaica, Malaya, New Guinea, Nigeria, North Borneo, Panama, Sierra Leone, Southern Cameroon, St. Lucia, Surinam, Trinidad, and Western Samoa, and from rubber in Ceylon, Ghana, and Malaya. *P. heveae*, used in inoculation tests and as a basonym by Orellana (4), was not included, for it is considered to be a species distinct from *P. palmivora*. Altogether, 235 isolates of the fungus were examined; it was possible to obtain only 10 cultures from rubber and the remaining 225 isolates were from cacao. Most cultures of the cacao pathogen were obtained from pod infections and the remainder were isolated from leaf, stem and flower cushion infections. Isolates were examined as to lesion development in cacao pods, sporangial dimensions, and oospore production. Uniform inoculation of the fungus into pods was made by using a cork borer to remove a tissue disc from each pod and filling the cavity with an equal sized piece of infected tissue from lesions in previously inoculated pods. Development took place at room temperature (27–30°C) in humid chambers of polythene sheeting over wire frames. Measurements were made of 100 sporangia of each isolate washed from pod surfaces 5–6 days after inoculation. Isolates from all countries and both hosts were intercrossed by growing paired cultures on 4% oatmeal agar at 15–16°C; at normal laboratory temperatures very few oospores were produced by any culture. In the examination all isolates were tested in the characters of the type of lesion produced in cacao pods and sporangial dimensions; for assessment of oospore production all isolates from within particular countries were inter-

crossed, and for crossing tests between countries selections of isolates from each country were grown in paired cultures.

Results.—No single isolate was found to produce oospores. The results of 1600 crossing tests, examined after 3 weeks for oospore production, showed that isolates from the different countries could be divided into 3 groups on the basis of complementary characters (Table 1). All crosses between isolates of Groups I and II resulted in production of oogonia with amphigynous antheridia, usually in abundance. The isolates listed in Group III were atypical in several respects and did not produce oospores when crossed with each other or with members of the other two groups. No paired cultures of Group I isolates were found to contain oospores at the end of the experimental period, but oospores were found in very occasional crosses between Group II isolates.

All isolates from both cacao and rubber infected cacao pods, and isolates from cacao in Ghana and Nigeria readily infected rubber pods. Cacao isolates from other countries were not tested in rubber pods. The type of lesion produced in cacao pods was variable; isolates of Group I produced lesions characterized by clearly defined regular margins, scattered sporangial masses, and a limited amount of aerial mycelium. Similar lesions were produced by the Group II isolates from Jamaica and North Borneo. Isolates from rubber in Ceylon and Malaya produced well defined lesions but very few sporangia. All Group II isolates from West African territories developed characteristic lesions with irregular and diffuse margins, a negligible amount of aerial mycelium, and dense, powdery sporangial masses. Lesions of Group III isolates were sharply defined but practically devoid of aerial mycelium and sporangia.

Differences were also noted between the groups in dimensions of sporangia from cacao pods. Isolates of Group I, except the Jamaican isolates and that from British Guiana, had slightly longer but appreciably narrower sporangia than those of Group II. Thus, the length-width ratios of the sporangia were almost invariably greater in the first group (1.7) than in the second (1.3), except the isolates from rubber (1.7). Dimensions of Group III isolates were similar to those of Group I but were of variable shape. Table 2 shows the dimensions of samples of 100 sporangia in washings from cacao pods after inoculation with isolates from cacao and rubber.

No differences could be distinguished between isolates from rubber and cacao in Ghana. They were identical in growth rate and in the type of lesion produced in cacao pods, had similar sporangial dimensions, and were incompatible with regard to oospore production; these findings agree with those of Robertson (6), who also examined the pathogen from rubber seedlings in Ghana. Cacao and rubber isolates from Ceylon, however, were complementary to each other, as were similar isolates from Malaya (A), except one rubber isolate (Malaya B), and isolates from both hosts developed strongly in cacao pods (Fig. 1). No major differences were found among cacao isolates from Ghana, Ceylon, and Malaya apart from small variations in growth rate in cacao pods. Lesion size in cacao pods infected with cacao and rubber isolates from the three countries are shown in Table 2, and results of crossing tests between isolates from the two hosts are summarized in Table 3.

Discussion.—Except for the observations of Ramakrishnan and Seethalakshmi (5) on homothallic iso-

Table 1.—Groupings of isolates of *P. palmivora* based on oospore production in paired culture by complementary strains. Isolates from cacao except where rubber specifically stated.

Group I	No. in group[a]	Group II	No. in group[a]
Belgian Congo	1/1	Cameroun	30/30
British Guiana	1/1	Ceylon-rubber	5/5
Ceylon	1/1	Fernando Po	7/7
Dominica	6/6	Gabon	3/5
(Gabon)[b]	2/5	(Jamaica)[b]	1/3
Ghana	57/57	Malaya-rubber	2/3
Ghana-rubber	2/2	Nigeria	42/47
Ivory Coast	7/7	North Borneo	1/1
Jamaica	2/3	Southern Cameroon	18/19
Malaya	2/2		
New Guinea	1/1		
(Nigeria)[b]	5/47		
Panama	3/3	Group III	
Sierra Leone	3/5		
(Southern Cameroon)[b]	1/19	Angola	1/1
St. Lucia	5/5	Malaya-rubber	1/3
Surinam	2/2	(Sierra Leone)[b]	2/5
Trinidad	1/1		
Western Samoa	5/5		

[a] Numerator represents no. of isolates falling within group; denominator represents total no. of isolates tested from each country.

[b] Isolates considered atypical of dominant pathogen type occurring within the country.

Table 2.—Average length of cacao pod lesions (cm) after 5 days, and mean length and width dimensions of 100 sporangia from infected cacao pods of cacao and rubber isolates of *P. palmivora*.

Isolate	Lesion length	Sporangia Length	Sporangia Width	Sporangial L:W ratio
Ghana-cacao	10.7	42.0	25.0	1.68
Ghana-rubber	11.2	40.6	24.8	1.64
Ceylon-cacao	9.5	43.0	25.4	1.69
Ceylon-rubber	10.0	44.5	26.0	1.71
Malaya-cacao	10.4	40.7	24.9	1.63
Malaya-rubber	9.6	42.5	25.0	1.70

Table 3.—Oospore production in paired cultures of *P. palmivora* isolates from cacao and rubber.

	Ghana–cacao							
Ghana-cacao	O	Ghana–rubber						
Ghana-rubber	O	O	Ceylon–cacao					
Ceylon-cacao	O	O	O	Ceylon–rubber				
Ceylon-rubber	X[a]	X	X	O	Malaya–cacao			
Malaya-cacao	O	O	O	X	O	Malaya–rubber A		
Malaya-rubber A	X	X	X	O	X	O	Malaya–rubber B	
Malaya-rubber B	O	O	O	O	O	O	O	

[a] X equals oospores produced in paired culture.

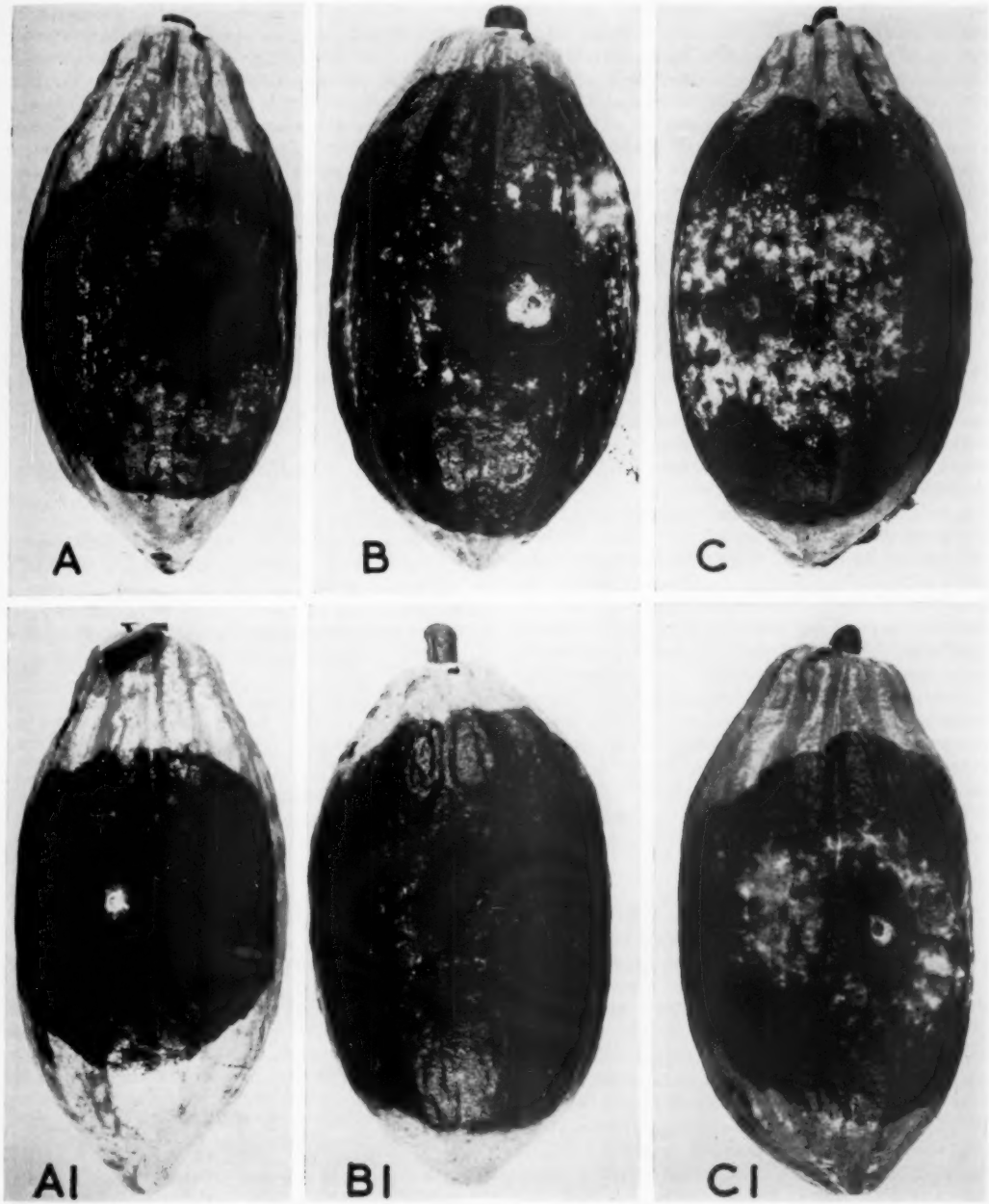

Fig. 1.—Isolates of *P. palmivora* from A Malaya, B Ceylon, C Ghana. A B C: isolates from cacao infecting cacao pods. A1, B1, C1: isolates from rubber infecting cacao pods.

lates of *P. palmivora* from *Areca catechu*, the species is often considered to be heterothallic. Gadd (2) and Ashby (1) reported major differences between isolates of the fungus from cacao, rubber, and other hosts, and that the main difference between isolates was the production of oospores only when complementary strains of the fungus were grown together. On this basis it was possible to subdivide the species into "cacao" and "rubber" groups, terminology which has remained in current usage although not implying a particular host specificity. Orellana (4) failed to find oospores in mixed cultures of isolates of the fungus taken from

cacao and rubber, and concluded that they were sexually incompatible. Moreover, he could not infect cacao pods with rubber isolates, and only incipient rotting followed inoculation of rubber pods with the cacao pathogen. On these criteria he proposed further subdivision of the species to include 2 new varieties, var. *theobromae* and var. *heveae*. The present study has not confirmed these observations; oospores were readily produced when paired cultures of complementary isolates from the two hosts were incubated at lower temperatures, as found by Ashby (1), and cacao pod infections by isolates from both hosts were induced without difficulty.

Survey of a large number of isolates has shown that "cacao" group isolates are found on rubber, and vice versa; in fact, the dominant pathogen in a large part of West Africa is of the "rubber" group. Hickman (3) reported that *P. palmivora* parasitizes plants of 51 genera in 21 families of flowering plants, and this in itself seems sufficient to preclude any subdivision of the species solely on the grounds of host specialization. It is evident that a number of strains of *P. palmivora* occur, as might be expected from the world-wide distribution of the pathogen.

All isolates of Group I are probably of the same strain, with slight variation between countries, although the British Guiana isolate differs in sporangial characters. Group II, however, is a heterogeneous collection of isolates; cultures from Jamaica and North Borneo resemble those of Group I but are sexually different, and rubber isolates from Ceylon and Malaya differ from others in the group in characteristic lesions in cacao pods. Yet another Malayan isolate was incompatible with all others. The Group II isolates from West Africa were quite unlike those from any other region in any group, and were exceptional in that very occasional oospore production was found in paired cultures. Although oospore production immediately distinguished between isolates of Groups I and II there was no sharp division between the two groups in other criteria. Furthermore, additional characters were necessary to subdivide Group II isolates. It is evident, therefore, that, even if the Group III isolates are excluded because they are possibly distinct from *P. palmivora*, at least 4 strains of *P. palmivora* are found on cacao and rubber alone. A complete comparative study of isolates from all known hosts of the fungus might reveal further strains and it may be possible to establish a varietal system of classification based on physiological and morphological data. —West African Cocoa Research Institute, Tafo, Ghana.

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THE USE OF FUSARIUM CULTURE FILTRATE MEDIA IN THE ISOLATION OF FUSARIA FROM SOIL

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SUMMARY

Media containing culture filtrates of *Fusarium solani* f. *phaseoli* were used to isolate that fungus from the soil in dilution plates. Colony counts on these media were comparable to those on modified Martin's medium. Restriction in both the numbers and growth of competing fungi was good, and the development of characteristic pigmentation and sporulation simplified the identification of *F. solani* f. *phaseoli* colonies. Filtrates from 2- to 3-week-old cultures gave the best results. Filtrates from older cultures inhibited *F. solani* f. *phaseoli* sufficiently to preclude quantitative studies. *F. oxysporum* f. *conglutinans* grew rapidly on its own filtrate medium, but its growth was markedly reduced on filtrate medium of *F. solani* f. *phaseoli*. This suggested that these filtrates were to some degree specifically selective. Pure-culture tests showed that many fungi were unable to grow on filtrate media of *F. solani* or *F. oxysporum*. Mycelium of *Phytophthora* sp. and *Pythium* sp. placed on the filtrate medium of *F. solani* failed to grow when returned to PDA. Filtrate medium diluted 50% and 75% with potato-dextrose broth retained the capacity to inhibit these fungi, but not completely. Autoclaving did not destroy the inhibitory properties of the filtrates. The use of culture filtrates may provide a useful technique for the assay of fungus populations in soil.

Determination of the presence and abundance of pathogenic fungi in soil provides valuable information

for predicting disease severity and assessing the effectiveness of soil treatments designed to reduce or eliminate such fungi. Recognition of the importance of the amount of inoculum in the epidemiology of root diseases has made desirable the development of techniques for estimating the populations of root-disease fungi in soil. A few special media have been developed for this purpose (3, 6). The effectiveness of these media results from the addition of materials that restrict the germination or growth of competing fungi while allowing satisfactory growth of the selected fungus on soil-dilution plates. Many fungi produce, *in vitro*, compounds that inhibit the growth of other fungi (1, 4, 7). Media prepared from culture filtrates of such fungi have been shown to reduce the growth of other fungi markedly (2, 5). The possibility was investigated that media prepared from culture filtrates might provide suitable substrates for selective isolation of certain soil fungi by the dilution plate method. The results of initial investigations are presented here.

Preparation of media and methods of study.—Culture filtrates were prepared by dispensing 200-ml lots of potato-dextrose broth into 16-oz. prescription bottles. These were then autoclaved, inoculated with spore masses from single-spore colonies of *Fusarium solani* f. *phaseoli* (Burk.) Snyd. & Hans., and incubated at room temperature for 14–16 days, unless otherwise indicated. These culture media were then filtered through a linen handkerchief and sterilized by passage through a Seitz filter. For special studies, similar filtrates were prepared of *F. oxysporum* f. *conglutinans* (Wy.) Snyd. & Hans.

Autoclaved filtrate media were prepared by adding Difco Bacto agar to the filtrates to give a 2% agar medium. These were then autoclaved for 15 minutes at 18 lb pressure. To test for possible inactivation of antibiotic compounds during autoclaving, solid media were prepared by dividing the filtrates into 2 lots. One lot, containing agar to give a 10% agar suspension, was autoclaved for 15 minutes. Four parts of the remaining lot, heated briefly to 50°C in an incubator, were then added to one part of the autoclaved lot to give a 2% agar medium. In this way only one-fifth of the medium was subjected to autoclaving. Just before these media were poured into Petri plates, 300 ppm of streptomycin sulfate were added. Modified Martin's medium was prepared according to the specifications of Snyder et al (6). All media used fell within the pH range of 4.8–5.5. This range was considered sufficiently narrow, and no adjustments were made.

Both field soils and fortified soil were used as inoculum for dilution plate studies. The fortified soil was a greenhouse soil mixture to which was added a dense conidial suspension of *Fusarium solani* f. *phaseoli*. Over a period of 2 years, 2 crops of beans were grown in this soil, which assured a dense population of *Fusarium* chlamydospores in the soil. This soil was used when high counts of *Fusarium* were desired for more precise comparison of media.

Soil suspensions were prepared by adding a weighed sample of soil to a known volume of 0.1% water agar. One ml of suspension was pipetted on to each plate and evenly distributed over the surface of the medium with a glass rod or by gentle agitation. After 5 or more days' incubation at room temperature, the plates were examined and the Fusarium colonies were counted.

Results.—Preliminary tests were made with filtrates of *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium*, *Fomes*, and *Armillaria* species. *Fusarium solani* f. *phaseoli* was selected for further study because of its inhibitory effect on other fungi and because there was available a medium (modified Martin's) for estimating soil populations of this fungus with which the effectiveness of filtrate media could be compared (6).

Comparison of filtrate media with modified Martin's medium.—Ten plates each of modified Martin's medium (MMM), autoclaved *Fusarium solani*-filtrate medium (AFSM), and unautoclaved *Fusarium solani*-filtrate medium (UFSM) were inoculated with a 1:4000 suspension of fortified soil. Colony counts of *F. solani* f. *phaseoli*, made after 6 days' incubation, averaged 12.8 colonies per plate on MMM, 13.5 on AFSM, and 11.9 on UFSM. These results indicated that colony counts on either filtrate medium were comparable to those on MMM. Differences were evident, however, in the growth of fungi on these media. On both AFSM and UFSM the colonies of *F. solani* f. *phaseoli* were more distinct, and characteristic pigmentation developed rapidly. Sporulation was good on both of the filtrate media and on MMM, but the colonies did not become pigmented on MMM. Thus the colonies were more obvious and more readily recognized on the filtrate media. Since the performance of AFSM was equal to that of UFSM, the latter was abandoned in subsequent experiments.

The performances of MMM and AFSM were also tested with soils from 2 bean fields to compare the effectiveness of these media for field studies. A 1:700 suspension was prepared from each soil, and 10 plates of each medium were inoculated from each suspension. In addition, 10 plates of AFSM were inoculated with 1:1400 suspensions of the two soils as a check on the reliability of the counts. After 6 days' incubation, colony counts of *F. solani* f. *phaseoli* from one soil were 2.1 per plate on AFSM and 1.3 per plate on MMM at 1:700, and the count on AFSM at 1:1400 was 1.1 per plate. With the other soil, corresponding counts were 1.0 per plate on AFSM and 1.3 per plate on MMM at 1:700, and 0.5 plate on AFSM at 1:1400. Thus the colony counts were similar on both media. In addition, the counts from the 1:700 and 1:1400 dilutions of each soil on AFSM were 2.1:1.1 and 1.0:0.5, indicating that the readings were close to a 2:1 ratio. Again the restriction of the growth of spreading fungi on AFSM was very good (Fig. 1). These results showed that the filtrate medium was dependably selective even at low dilutions of field soils.

The effect of incubation period on filtrate activity.—Previous tests were made only with filtrates from 2-week-old cultures. Filtrates from cultures of different ages were tested to determine the incubation period necessary to produce the best filtrate medium for the isolation of *F. solani* f. *phaseoli*. During 4 weeks, bottles of potato-dextrose broth were inoculated at one-week intervals with *F. solani* f. *phaseoli*. Autoclaved filtrate media were prepared from the resulting cultures, and 10 plates of each medium were inoculated with a 1:3000 suspension of fortified soil. The results are shown in Table 1. On filtrate medium from 1-week-old cultures, the total number of fungus colonies was high, the *F. solani* f. *phaseoli* count was reduced, and the growth of spreading fungi was rapid. The total colony counts, the *F. solani* f. *phaseoli* counts, and the restriction of the growth of spreading fungi were similar on filtrate media from either 2- or 3-week-old cultures. On the filtrate medium from 4-week-old cultures, the total colony count, the *F. solani* f. *phaseoli* count, and the growth of all fungi were markedly reduced (Fig. 2). These results indicated that 2–3 weeks' incubation of cultures gave the most consistent and satisfactory filtrate medium. Four weeks' incubation gave greater inhibition of other fungi, but the reduced colony count of *F. solani* f. *phaseoli* indicated that quantitative data were not satisfactory.

Table 1.—Average colony count of *Fusarium solani* f. *phaseoli* on filtrate media prepared from cultures of 4 ages and inoculated with a suspension of fortified soil.[a]

	Av. count per plate with filtrate medium from culture of age indicated (weeks)			
	1	2	3	4
Fungus colonies	73.8	48.0	53.0	37.6
F. solani f. *phaseoli* colonies	15.4	19.0	19.0	11.4
Fungus colonies greater than 1 cm in diameter	4.0	1.8	2.0	0.0

[a] Averages are based on counts from 10 plates of each medium.

Comparison of filtrate media of F. solani and F. oxysporum.—In comparing plates of MMM with AFSM, it was noted that the growth of *F. oxysporum* was greatly reduced on AFSM. This observation suggested that the selectivity of filtrate media might be somewhat specific. For testing this possibility, AFSM and a similar medium (AFOM) prepared from cul-

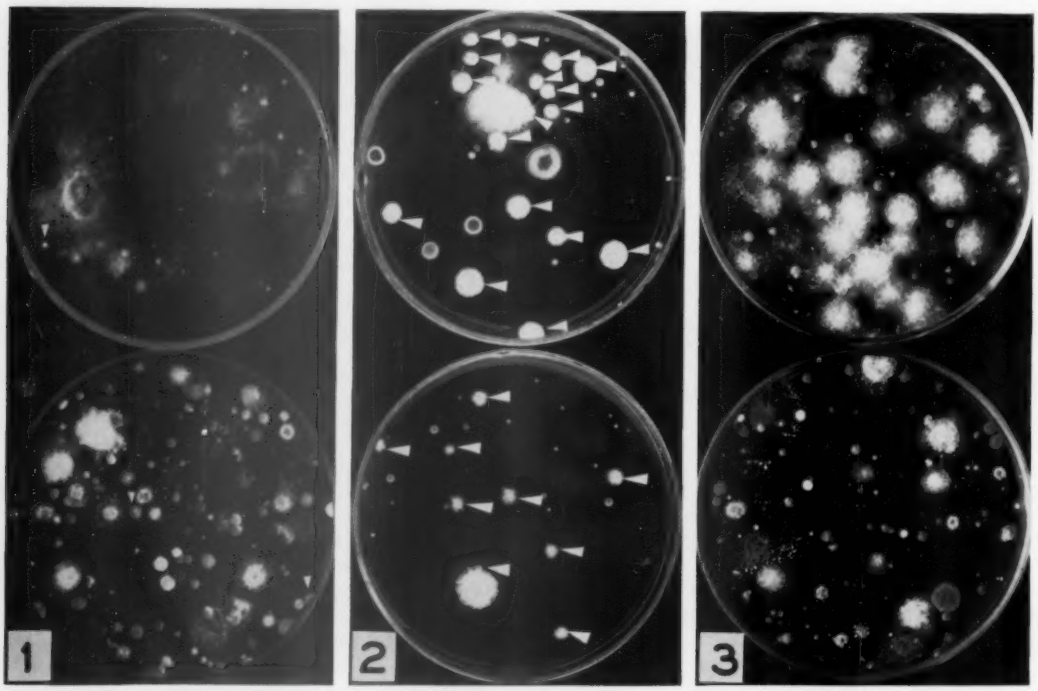

Fig. 1–3.—1) Soil dilutions plated on modified Martin's medium (above) and on *Fusarium solani*-filtrate medium (below) showing the differences in growth of spreading fungi on the 2 media. The small white arrows indicate colonies of *F. solani* f. *phaseoli*. **2)** Soil dilutions plated on *F. solani*-filtrate medium prepared with filtrates from 2-week-old cultures (above) and 4-week-old cultures (below) showing the differences in numbers and growth of *F. solani* f. *phaseoli* on the 2 media. Small white arrows indicate colonies of *F. solani* f. *phaseoli*. **3)** Soil dilutions plated on *F. oxysporum*-filtrate medium (above) and *F. solani*-filtrate medium (below) showing the differences in the numbers and growth of *F. oxysporum* colonies on the 2 media. Rapid growth of numerous *F. oxysporum* colonies has nearly covered the plate of *F. oxysporum*-filtrate medium, whereas both the number and the growth of *F. oxysporum* colonies is greatly reduced on the *F. solani*-filtrate medium.

tures of *F. oxysporum* f. *conglutinans* were inoculated with 1:200 and 1:1000 suspensions of bean-field soil and of greenhouse soil containing *F. oxysporum* f. *conglutinans*. Ten plates of each medium were inoculated for each dilution of each soil. Colony counts of *F. solani* f. *phaseoli* from the bean-field soil averaged 1.9 and 0.4 per plate, respectively, at the 1:200 and 1:1000 dilutions on AFSM, and 0.5 colonies per plate at the 1:1000 dilution on AFOM. Colony counts could not be made from the 1:200 dilution plates of bean-field soil on AFOM, because of the rapid growth of *F. oxysporum*. No colony counts were made of *F. oxysporum* f. *conglutinans*, because no suitable method was available for distinguishing this form from other forms of *F. oxysporum*. Differences in the growth of *F. oxysporum* on the two media were obvious (Fig. 3).

The growth of *F. oxysporum* colonies was markedly reduced on AFSM, thus allowing the examination of individual colonies of slow-growing fungi such as *F. solani* after 10 or more days' incubation. AFOM, in contrast, allowed such rapid growth of *F. oxysporum* colonies from either soil that slower-growing fungi were quickly obscured. These results indicated that there were important differences between the filtrates of these 2 Fusaria and that different filtrate media might be valuable for selective isolation of various species of *Fusarium*.

The reciprocal effects of AFSM and AFOM were further investigated by determining the growth rates of each fungus on 10 plates of its own filtrate medium, 10 plates of the filtrate medium of the other fungus, and 10 plates of PDA. Growth from hyphal transfers placed in the center of 5-cm plates was measured at 2-day intervals for 10 days or until the colonies filled the plates. The results are in Fig. 4.

The growth curves for the 2 fungi on the 3 media showed that *F. oxysporum* f. *conglutinans* grew nearly as well on its own filtrate medium as it did on PDA. Its growth was greatly reduced on AFSM. The growth of *F. solani* f. *phaseoli* was similar on either filtrate medium, both allowing less growth than PDA. Thus the filtrate media of these 2 fungi had a similar effect on *F. solani* f. *phaseoli* but differed greatly in their effects on *F. oxysporum* f. *conglutinans*.

The effects of Fusarium filtrate media on the growth of other fungi in pure culture.—The effects of AFSM on the growth of various fungi in pure culture were tested to determine the types of fungi inhibited and to investigate the properties of such media further. Hyphae from actively growing colonies of each test fungus were planted in the center of five 5-cm plates containing AFSM and 5 similar plates of PDA. Growth rates on AFSM and on PDA were compared over periods of 10–25 days.

The results on AFSM were as follows. No growth: *Phytophthora cinnamomi* Rands, *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, *Phythium ultimum* Trow., *Mucor ramannianus* A. Moeller, *Mortierella* sp., *Endothia parasitica* (Murr.) A. & A., *Cytospora* sp., *Pestalotia* sp., *Ramularia* sp., *Fomes annosus* (Fr.) Cke., *Armillaria mellea* (Vahl.) Quel., *Boletus variegatus*

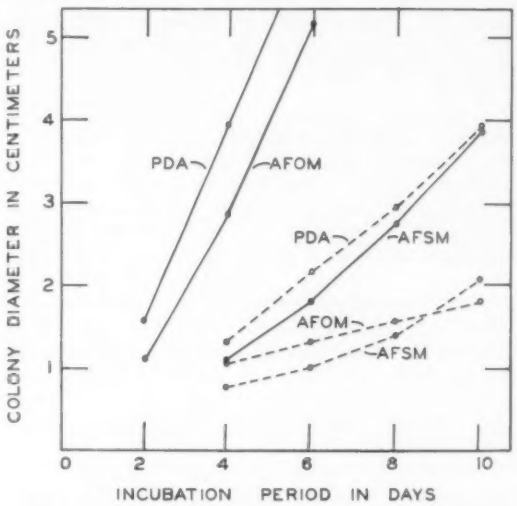

Fig. 4.—Growth rates of *Fusarium oxysporum* f. *conglutinans* (solid lines) and *F. solani* f. *phaseoli* (broken lines) on PDA, AFOM, and AFSM.

Schwartz ex Fr., *B. bovinus* L. ex Fr., *B. granulatus* L. ex Fr., *Amanita muscaria* Fr., and *Coprinus commatus* (Muller ex Fr.) Gray. Growth reduced 75% or more: *Sclerotium bataticola* Taub., *S. rolfsii* Sacc., *Verticillium albo-atrum* Reinke & Berth., *Gliocladium* sp., *Trichoderma* sp., and *Rhizoctonia solani* Kühn. Growth reduced 50% or more: *Colletotrichum gloeosporioides* Penz., *Mycelium radicis atrovirens*, *Cephalosporium* sp., and *Phialophora* sp. Growth reduced less than 50%: *Chaetomium* sp., *Zygorhinchus* sp., and *Thielaviopsis basicola* (Berk.) Ferr.

Similar studies were made with AFOM on a smaller number of fungi. No growth: *Phytophthora cinnamomi*, *Pythium ultimum*, *Mortierella* sp., *Thielaviopsis basicola*, *Coprinus commatus*, and *Armillaria mellea*. Growth reduced 75% or more: *Mucor ramannianus*, *Pestalotia* sp., *Fomes annosus*, and *Rhizoctonia solani*. Growth reduced 50% or more: *Sclerotium rolfsii*. Growth reduced less than 50%: *Trichoderma* sp. and *Sclerotium bataticola*. Thus the inhibition of most fungi by the 2 media was similar but not identical, as indicated by the reaction of *Trichoderma* sp. and *S. bataticola*. These observations indicate that there are both significant similarities and significant differences in the activity of the filtrate media of these 2 species of *Fusarium*, and that a wide range of fungi are inhibited.

The antifungal activtiy of AFSM was investigated further by tests in which *P. ultimum* and *P. citrophthora* were grown on full-strength AFSM, on AFSM diluted with potato-dextrose broth to 50% and 25% strength, and on PDA. Colony diameters of each fungus on 8 plates of each medium were measured at 7 and 14 days. Direct comparison of growth on the filtrate media with that on PDA could not be made,

since both fungi covered the plates of PDA before measurable growth occurred on the filtrate media. Differences in growth on the 4 media were obvious, however.

Neither fungus grew on the full-strength or 50%-strength AFSM. On 25%-strength AFSM, colonies of *P. citrophthora* averaged 1.4 cm at 14 days, and colonies of *P. ultimum* averaged 3.0 cm at 7 days and filled the plates by 14 days. Thus the filtrate medium completely inhibited growth of the test fungi at 50% strength, and growth was greatly reduced at 25% strength.

Further indication of the potency of AFSM was obtained by planting hyphae of *P. ultimum* and *P. citrophthora* on the medium for 10 days and then returning the hyphae to PDA. Of the 10 transfers of each fungus so treated, none grew when returned to PDA. These results suggest that highly fungicidal materials were present in the filtrate medium.

Discussion.—Many fungi produce antibiotic materials in culture (1, 4, 7). The survival value of this capacity undoubtedly rests with the ability of the fungus to tolerate greater concentrations of these materials than can competing microorganisms. This ability also provides a logical basis for the development of selective media, as indicated by experiments with culture filtrates of *Fusarium* species.

The growth of both *F. solani* f. *phaseoli* and *F. oxysporum* f. *conglutinans* modified potato-dextrose broth in such a way that solid media prepared from culture filtrates were entirely unsuitable for the growth of many fungi, and the growth of many other fungi was greatly reduced. This inhibition of competing fungi on soil-dilution plates prepared with filtrate media allowed consistent estimation of the populations of *F. solani* f. *phaseoli* in soil.

The selective capacity of *Fusarium* filtrate media indicates that the two species tested produce, *in vitro*, materials active against a wide range of fungi. Comparison of filtrate media from cultures of *F. solani* f. *phaseoli* and *F. oxysporum* f. *conglutinans* suggests that more than one material was involved, since these media had a similar effect on some fungi and a dissimilar effect on others. In addition, the growth of *F. solani* f. *phaseoli* was similar on either medium, whereas the growth of *F. oxysporum* f. *conglutinans* was greatly reduced on the filtrate medium of *F. solani* but not on its own filtrate medium. These differences in the growth of the 2 species on the filtrate media provided a convenient method for selectivity isolating either species from soil.

Filtrate media of *Fusarium* species offer distinct advantages for use in the isolation of Fusaria: their preparation is simple. The antifungal materials are heat-stable. Solid media can be prepared in large quantities and stored in stock bottles for future use. Restriction of both numbers and growth of extraneous fungi is very good. Sporulation and pigmentation on the media aid in the identification of *Fusarium* colonies.

The results obtained with filtrate media of Fusaria suggest that results might be similar with filtrate media of other fungi possessing the capacity to produce antibiotic materials. By combining the filtrates of 2 or more fungi, it may be possible to eliminate greater numbers of unwanted fungi from isolation plates. It also appears feasible to use filtrates as basic media to which selectively antifungal compounds may be added, thus allowing the natural inhibitory capacity of a fungus to augment the activity of the added materials. In addition, the filtrate medium of any one fungus could be used to isolate not only that fungus but any fungus able to tolerate the filtrate.—Department of Plant Pathology, University of California, Berkeley 4.

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BOOK LISTING

FERGUS, CHARLES L. 1960. Illustrated genera of wood decay fungi. Burgess Publishing Company, Minneapolis. 132 p. Price: $4.00.

Wood-decaying fungi in 85 genera in 10 families are described in this loose-leaf manual. Nearly all genera are illustrated by photographs of fruiting bodies of representative species. In addition to descriptive text there are some 30 keys to families, genera, and species.

INFLUENCE OF SOIL TEMPERATURE, ISOLATES, AND METHOD OF INOCULATION ON RESISTANCE OF MINT TO VERTICILLIUM WILT

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SUMMARY

Field tests demonstrated that *Mentha piperita*, *M. spicata*, *M. piperita* × *M. crispa* hybrids 148, 161, and 185, and *M. crispa* possessed an inherent range of increasing resistance to *Verticillium albo-atrum*. Rooted cuttings of these 6 clones when inoculated and grown in soil infested with *Verticillium albo-atrum* in a 19–26°C greenhouse had greatest disease severity for the first 4–6 weeks when soil temperature was 25°. Most clones later recovered at 25°, and toward the latter part of the test periods disease was greater at 20°. The tendency for recovery was still more pronounced at 30°. Diseased plants in 10° soil were characterized by slow but increasing disease, but growth of the pathogen in culture was almost equally limited at 10° and 30°C. Unrooted cuttings inoculated by immersing the cut ends in inoculum for 48 hours showed a range of resistance similar to that of root-inoculated cuttings, but the over-all disease intensity was less. When wounded stems were inoculated, differential resistance was also expressed. Isolates that were only mildly virulent in root inoculations were more virulent when used in stem wound inoculations. It was concluded that resistance was common to stem and root but the level was greater in the stem. By controlling the soil temperature and choosing the inoculation method, 6 levels of resistance could be identified in the greenhouse.

Verticillium wilt causes serious losses in mint. Control measures such as deep-plowing, crop rotation, and use of chemical soil amendments and antagonistic organisms are only partially or temporarily effective (3, 11). Plant resistance has not been used for control because of the critical flavor requirements of the mint-oil industry. Economic considerations, however, may force acceptance of resistant varieties if losses from wilt increase.

Mentha crispa L., a poor-quality mint with relatively high wilt resistance, has long been used in crosses with commercial peppermint, *M. piperita* L., to develop resistant peppermint types (11). Most of the few highly resistant hybrids produced by this cross usually resemble *M. crispa* in vegetive appearance and have oils of poor quality, whereas some hybrids with intermediate resistance approach *M. piperita* more closely in appearance and oil quality. Sterility has seriously limited the breeding procedures normally used to transfer resistance. Present evidence indicates that intermediate resistance may be adequate for commercial production, and certain selections of *M. spicata* L., with better oil quality than that of *M. crispa*, are being used as a source of resistance. It is possible to determine high and low resistance in the field and greenhouse, but no effective screening technique has been available for determining intermediate levels of resistance. This obstacle has prevented a thorough understanding of the mode of inheritance of wilt resistance.

This paper reports results of certain tests made in developing a method of identifying different levels of resistance.

Materials and methods.—*Mint clones.*—Field tests in Indiana on muck naturally infested with *Verticillium albo-atrum* Rke. & Bert. were conducted in 1948–1955 with *M. piperita*, *M. spicata*, *M. crispa*, and numerous hybrids of *M. piperita* × *M. crispa*. Resistance of each clone was determined from the prevalence and severity of symptoms exhibited by 10 or more plants in each of 4 replications. Six clones selected from these tests were compared for resistance in replicated tests at 2 locations in 1956-58. Wilt ratings indicated that these clones (*M. piperita*, *M. spicata*, *M. piperita* × *M. crispa* hybrids 148, 161, and 185, and *M. crispa*) possessed an inherent range of increasing resistance.

Cultures.—Seven isolates of *Verticillium* were used. Unless otherwise indicated, isolate I1 was used. Isolates I1, I2, and I3 were from single spores of a culture of *V. albo-atrum* from infected mint at the Purdue Muck Crops Farm, Walkerton, Indiana. These 3 isolates represented the predominant culture types among 75 single-spore isolates studied. Isolate M was from mint collected at Mentha, Michigan, and isolate 0 from infested soil collected at Jefferson, Oregon. Isolate P92 was supplied by C. E. Horner, Corvallis, Oregon, and isolate V22 by R. E. Webb, Beltsville, Maryland.

Greenhouse studies.—The 6 mint clones were maintained in soil beds in the greenhouse. Cuttings from vigorously growing shoots were rooted in Perlite. Two-week-old cuttings were used in all tests where roots were inoculated. The plants were grown in soil in 4- and 6-in. clay pots. For tests with constant soil temperatures of 10, 20, 25, and 30°C, 1-gal. crocks were used in controlled-temperature water tanks. To prevent excessive runner growth, supplemental light was provided from midnight to 3:00 A.M.

All inoculum was grown on cellophane on CDA to avoid incorporation of large amounts of organic matter into the soil. Several methods of inoculation were used: 1) dipping roots in homogenized mats and transplanting in steamed soil; 2) transplanting similarly inoculated cuttings in artificially infested soil; 3) immersing ends of unrooted cuttings for 48 hours in an agitated suspension of inoculum; and 4) inserting inoculum into a cut in stems of 5-week-old plants at the soil level.

All tests were carried out in a 19–26°C greenhouse. At least 6 rooted and unrooted inoculated cuttings of each clone were studied in at least 4 trials. For tests in which wounded stems were inoculated, at least 9

plants of each clone were used in each treatment in 2 trials. Equal numbers of control plants were maintained for all treatments.

A disease-severity index was based on the percentage of plants showing symptoms and the intensity of symptoms, particularly stunting. An index from 0 (no disease) to 5 (severe disease) was derived. Stunting was less subject to variability than green or dry weight. Variability was found to be greater for inoculated plants than for uninoculated (Table 1). Disease ratings and height measurements were made at 2-week intervals for 8 weeks after inoculation. Attempts were made to relate root damage and disease severity by weighing fresh and dry roots, but this measure proved more variable than height measurement or foliage weight, primarily because of the difficulty in recovering the decayed portions of the root (Table 1).

Table 1.—Coefficients of variability for mint clones uninoculated and inoculated with *V. albo-atrum* and grown in soil at 3 temperatures.[a]

Soil temp. (°C)	Plant height	Foliage		Roots	
		Green weight	Dry weight	Green weight	Dry weight
Uninoculated					
10	15	29	28	55	41
20	8	25	25	42	47
30	6	31	30	60	52
Inoculated					
10	20	54	51	65	56
20	21	48	43	49	45
30	18	86	83	79	61

[a] Each figure is an average for the mint clones *M. piperita*, PC148, and PC185.

Results.—*Effect of soil temperature.*—1) *Root inoculation.*—In preliminary tests for resistance, in clay pots in the greenhouse, the levels of resistance of the same clones differed widely. A constant soil temperature reduced the variability, however.

In artificially infested soil *M. piperita* was the most severely diseased of the 6 clones at all temperatures. Disease was initially greatest at 25°C, but 4–6 weeks after inoculation became greater at 20°C. At 10° disease was slow to develop but gradually increased until it approached the intensity expressed at 20 and 25°. Disease development was high at 30°, though below that at 20 and 25°. Similarly in *M. spicata* and PC148, although disease development was initially more rapid at 25 than at 20°, 4 weeks after inoculation severity became greater in plants at 20°C. In plants grown at 10 and 30°, the disease was initially higher at 30° but after 6 weeks higher at 10°. From Fig. 1 it can be seen that over-all disease severity could be rated in decreasing order from *M. piperita* to *M. spicata* to PC148. PC161, PC185, and *M. crispa* exhibited markedly less disease severity than the other 3 clones and could be ranked in that order for increasing resistance. In general these clones exhibited slow disease development and rapid recovery. This phenomenon was observed by Wilhelm (14) in resistant strawberry clones infected with *V. albo-atrum*.

Disease development in root-inoculated cuttings was less intense in transplants to steamed soil than transplants to infested soil. Therefore, it was more difficult to distinguish the higher levels of resistance at all temperatures. Eight weeks after inoculation, *M. piperita* showed marked disease severity at 20° and 25°C. At 30° *M. piperita* showed marked disease intensity after 4 weeks but later tended to recover. At 10°, symptoms in *M. piperita*, although delayed in appearing, continued to increase in intensity but did not reach the same intensity as at 20 and 25°. At 20 and 25° *M. spicata* showed slight wilt symptoms 4 weeks after inoculation but had recovered by the end of the 8-week period. In steamed soil *M. piperita* could be readily differentiated from the other 5 clones.

2) *Inoculation of unrooted cuttings.*—Inoculation of unrooted cuttings at a soil temperature of 20°C resulted in the expression of the expected levels of resistance by the 6 clones, but in general the level of disease severity did not reach that of cuttings whose roots were inoculated. Two weeks after inoculation, *M. piperita* and *M. spicata* showed moderate disease, PC148 and PC161 showed a trace, and PC185 and *M. crispa* were free of symptoms (Table 2). The symptoms on *M. piperita* later intensified, whereas *M. spicata* showed very little increase in disease, and had considerably recovered by the end of the 8-week test period. PC148 and PC161 showed little further increase in disease, and had almost completely recovered by the end of the tests. PC185 and *M. crispa* remained almost free of symptoms. Unrooted cuttings were also inoculated at soil temperatures of 10, 25, and 30°C. Trends in disease development in the 6 clones were similar at these temperatures as at 20°C. At 10°C, however, there was a tendency for a delay in symptom expression as well as in recovery. At 25 and 30°, symptom expression was initially enhanced and recovery from disease was more rapid.

Root and stem inoculation with other Verticillium isolates.—1) *Cultures.*—The isolates differed primarily in degree of microsclerotium production. In particular, I2 and I3 produced fewer microsclerotia (Fig. 2). For study, microsclerotia and conidia from 2-week-old cultures were mounted in cotton-blue in lactophenol. Measurement of 100 microsclerotia from each of cultures I1, I2, I3, M, O, P92, and V22 showed that average microsclerotial diameter ranged from 8.9 to 9.2 μ. Measurements of 100 conidia from 14-day-old cultures of each of the 7 isolates, however, showed consistent differences between the isolates, permitting their separation into groups associated with source. Thus, isolates I1, I2, I3, and M, all from the Midwest, respectively, had the following dimensions (μ): 2.8 × 5.2, 2.8 × 5.3, 2.7 × 5.1, and 2.9 × 5.3; isolate V22, from Maine, 3.2 × 5.8; and isolates P92 and O, from Oregon, 4.1 × 7.0 and 3.6 × 6.3.

Optimal temperatures for growth were determined by inoculating CDA (Czapek-Dox-agar) medium in Petri plates with 0.5-cm-diameter portions from 2-

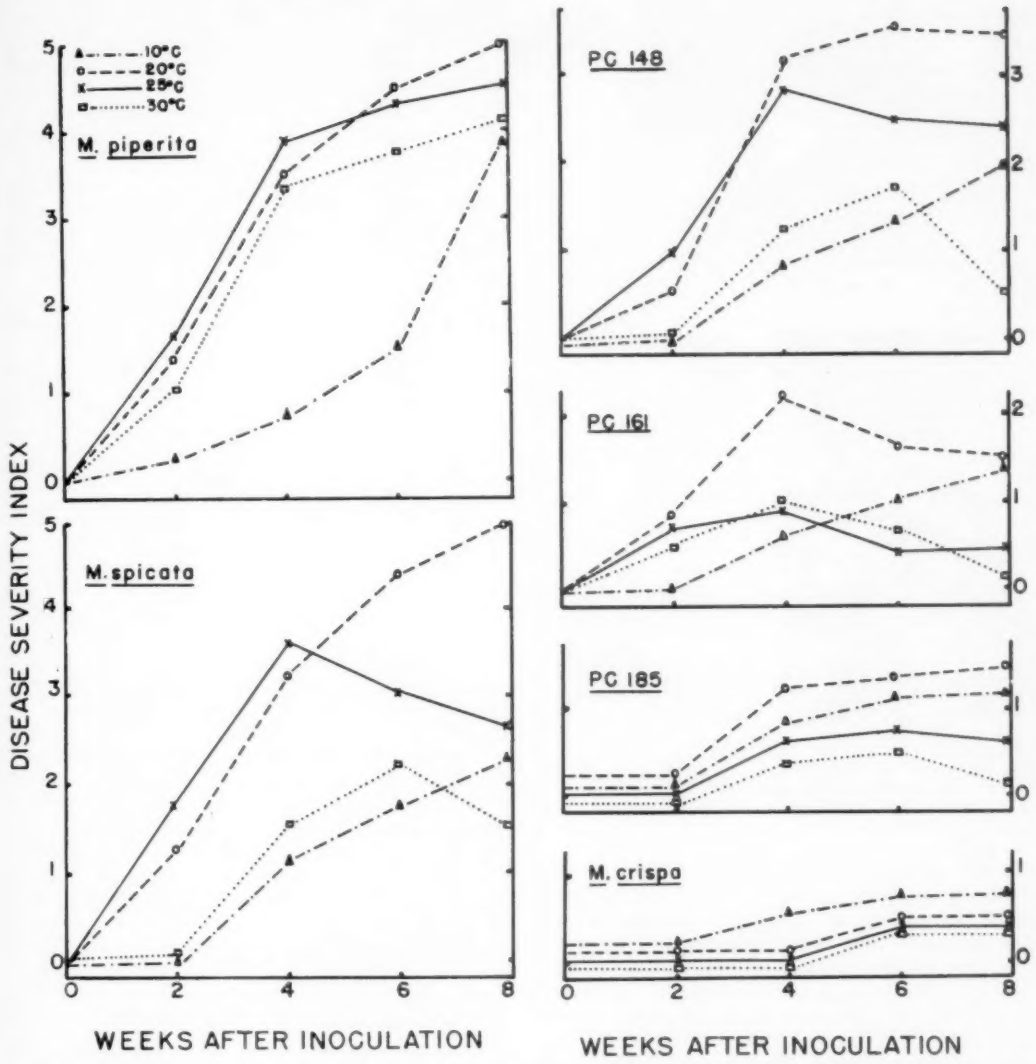

Fig 1.—Effect of soil temperature on disease severity for 8 weeks after inoculation with *V. albo-atrum*. The roots of 6 clones with different degrees of resistance were planted in infested soil. For all 4 temperatures over-all disease severity could be rated in decreasing order from *M. piperita* to *M. spicata* to PC148. PC161, PC185, and *M. crispa* exhibited disease development but rapid recovery.

week-old cultures. The isolates were grown 14 days at 6, 10, 15, 20, 22.5, 25, 27.5, 30, 32.5, and 35°C. Each isolate was grown on 4 plates at each temperature in 2 trials. The temperature optima for growth of the 7 isolates ranged from 22.5 to 27.5°C. Isolates I1, I2, I3, M, and O had a growth optimum of 22.5–25°, whereas P92 and V22 had one of 25–27.5°. The last 2 isolates also grew more at temperatures approaching the extremes for growth, that is, 6, 10, 30, and 32.5°.

2) *Root inoculation.*—When *M. piperita*, PC148, and PC185 were doubly inoculated by being root-inoculated and grown separately in soil infested with each of the 7 isolates, differences in virulence were marked. On *M. piperita*, I1 and M were highly virulent, O, I2, and I3 were moderately virulent, and P92 and V22 produced no visible disease symptoms. On PC148 and PC185, I1 and M were moderately virulent, O, I2, and I3 were slightly virulent, and P92 and V22 again produced no visible symptoms.

3) *Stem inoculation.*—When the stems of *M. piperita*, PC148, and PC185 were inoculated with the 7 isolates, rates of appearance of symptoms were similar for isolates I1, M, O, I2, and I3 (Fig. 3). Subsequently, isolates I1 and M produced the greatest disease

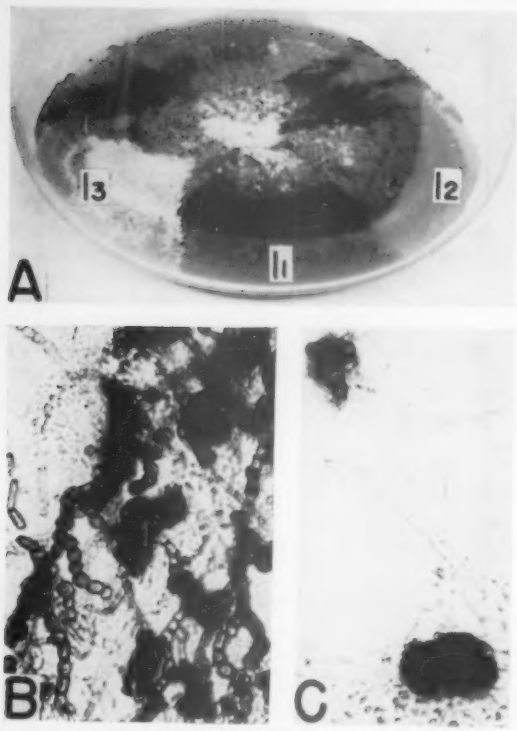

Fig. 2.—**A)** Sectoring in an isolate of *V. albo-atrum* from mint, the source of monosporic isolates I1, I2, and I3. **B)** Typical microsclerotium formation by I1 (×200). **C)** Sparse clumped type of microsclerotium formation by I2 (×200).

severity, and isolates O and I2 were less virulent. Isolate I3 was only mildly virulent. The levels of disease severity for these isolates were greater from stem inoculation than root inoculation. The sequence of virulence of the isolates remained the same through each test. Disease intensity continued to increase to the end of the test period in *M. piperita*, whereas PC148 and PC185 showed a marked tendency to resume vigorous growth at the end of the test period. Isolates P92 and V22, as was also true where these were tested by root inoculation, produced no visible disease on any of the clones. These 2 isolates were stimulatory, and the inoculated plants were more vigorous and taller than the checks. As far as we are aware, this is the first observation of stimulation in mint artificially inoculated with *V. albo-atrum* and it appears to be similar to the "bakanae effect" produced by certain species of *Fusarium* (1).

Discussion.—Soil temperature markedly affected expression of resistance, and, if sufficiently favored, the pathogen could cause severe wilt symptoms in the more resistant clones. Optimum temperature for disease intensity was closely related to that for growth of the pathogen in culture. However, this relation was generally applicable only during the initial phases of the test periods. Thus, although the optimum temperature for growth of the pathogen is 22.5–25°C in culture, and the optimum soil temperature for disease development was initially 25°, toward the latter part of the test period disease intensity became greater at 20°. This agrees with previous studies on the susceptpathogen relation of *Verticillium* (6, 8, 10). Thus, where suscept growth was favored over that of the pathogen, mint clones, particularly those with high resistance, tended to recover. Such recovery was more pronounced at temperatures closer to the growth limits

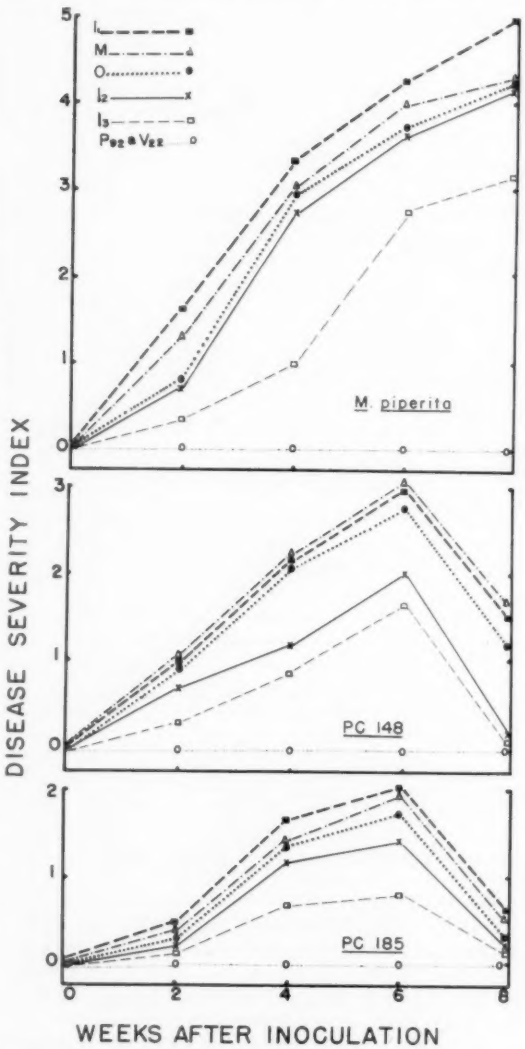

Fig. 3.—Disease severity in 3 mint clones for 8 weeks following stem wound inoculation with 7 different *V. albo-atrum* isolates. Symptoms appeared at the same rate for isolates I1, M, O, and I2. I3 was only mildly virulent. In *M. piperita* disease intensity increased while PC148 and PC185 recovered. Isolates P92 and V22 produced no disease.

Table 2.—Intensity of disease[a] in mint clones inoculated with *V. albo-atrum* prior to rooting and grown at 20°C soil temperature.

Clone	Time after inoculation			
	2 weeks	4 weeks	6 weeks	8 weeks
M. piperita	3.1	3.3	3.9	4.0
M. spicata	2.9	3.0	2.7	1.6
PC148	0.8	1.1	1.1	0.3
PC161	0.5	0.5	0.8	0.0
PC185	0.0	0.3	0.8	0.0
M. crispa	0.0	0.0	0.5	0.0

[a] Disease-severity index based on percentage of plants showing symptoms and intensity of symptoms (0, no disease, to 5, severe disease).

Table 3.—Radial growth (mm) of isolates of *V. albo-atrum* after 2 weeks[a] at 10 temperatures (°C).

Isolate	6	10	15	20	22.5	25	27.5	30	32.5	35
I1	6	16	33	46	51	50	48	20	5	0
I2	3	15	32	49	50	55	48	20	5	0
I3	3	13	30	45	49	58	43	13	2	0
M	4	16	32	48	52	52	46	16	3	0
O	7	17	32	45	53	53	52	18	5	0
P92	10	20	38	50	55	61	57	26	10	0
V22	8	18	33	47	53	58	55	25	7	0

[a] Average for 4 plates in each of 2 trials.

of the pathogen. For example, diseased plants in 10° soil were characterized by slow but increasing disease. On the other hand, although growth of the pathogen in culture at 10° and 30° was almost equally limited, inoculated plants at 30°, which initially showed wilt symptoms, were characterized by their ability to recover from the disease. The marked effects of different soil temperatures on disease development indicated the importance of the roots in determining differential resistance. The results of tests in which unrooted cuttings were inoculated indicated that resistance levels were similar in stem and root. It can be questioned, however, whether the method used truly inoculated the stem since the stem portion below the inoculum suspension functions as a root (7). However, levels of resistance were distinguishable by inoculation of wounded stems. Horner (5) presented evidence that the site of differential resistance to *Verticillium* is located in the stems as well as in the roots rather than just in the roots, as suggested by Keyworth (7). Our data are in agreement with those of Horner. We agree with Keyworth, however, that resistance in the stems is high, regardless of levels of resistance. Some disease development occurs in the root-inoculated clones with very high levels of resistance. Thus, if stem resistance was low, infection should be sufficient to cause marked symptom expression in all the clones. However, the opposite is true. Only in a very susceptible clone like *M. piperita*, in which infection via the root system is severe, is foliage symptom expression intense.

Although soil temperature and method of inoculation can result in marked differences in disease expression by the clones, other environmental factors are also important. Thus, although tests were repeated in the same greenhouse at a temperature range of 19–26°C, it was found that disease intensity was generally lower in midwinter than in spring or fall. The greatly reduced light intensity in winter at Beltsville essentially results in shaded plant growth. Roberts (12) reviewed the suppression of *Verticillium* by shade. Suppression of wilt symptoms was attributed to low carbohydrate production. Selman and Buckley (13) recently found an association between low sucrose in inoculum and low inoculum potential of *Verticillium*. The effects of radiation and seasonal change on the development of Verticillium wilt was recently studied (2, 4). Flowering and its resultant initiation of physiologic maturity also altered expected resistance levels. This complicating factor can be partly avoided by the use of freshly rooted cuttings from vigorously growing plants. Physiologic maturity and its relation to Verticillium wilt development have been reported (9, 10, 11). In general, physiologic maturity is associated with high carbohydrate levels and accelerated disease development. Maturity would have to receive careful consideration in selection for Verticillium resistance since there could be selection of late flowering clones which lacked the inherent resistance desired.

The studies reported provide the basis for an effective means for differentiation of the various levels of wilt resistance in mint under controlled conditions. In addition, results are directly applicable in explaining the marked variation in the expression of this disease during epiphytotics under different environmental conditions.—Agricultural Research Service, Crops Research Division, Beltsville, Maryland.

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RESISTANCE TO THE WILDFIRE DISEASE IN RELATION TO THE GENE-CHROMOSOME SET RATIO IN TOBACCO

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The investigation is part of a project of The Kentucky Agricultural Experiment Station, and published with approval of the Director. Accepted for publication August 27, 1960.

SUMMARY

The relationship was inverse between the number of genes present for resistance to wildfire and the population of *Pseudomonas tabaci* within the 2x, 3x, and 4x tobacco plants. The bacterial population was greatest in plants having no genes for resistance and least in plants having a gene-x ratio of 2/2, 3/3, and 4/4, whereas the population was intermediate in plants with a gene-x ratio of 1/2 and 2/4. As the number of viable bacteria recovered increased, disease symptoms became more severe.

Burley tobacco (*Nicotiana tabacum* L. n = 24) with resistance to wildfire, *Pseudomonas tabaci* (Wolf & Foster) Stevens, has been developed by transferring resistance from *Nicotiana longiflora* Cav. (n = 10) (1, 2, 6). Diachun and Troutman (3) showed that *N. longiflora* and *N. tabacum* plants with *N. longiflora* resistance would support bacterial multiplication, but at a lower rate than susceptible *N. tabacum*. Stokes (5) determined the relative rate of multiplication of *Ps. tabaci* in susceptible tobacco Ky 16, resistant Burley 21 and F_1 Ky 16 × Burley 21. Multiplication was found to be greatest in the susceptible, least in the resistant, and intermediate in the hybrid.

The present study was made to determine the population of *Ps. tabaci* in 2x, 3x, and 4x *N. tabacum* plants with 0, 1, 2; 0, 1, 2, 3; and 0, 2, and 4 genes for resistance to wildfire.

Methods.—Tetraploids were produced from Burley 21 and Ky 16 by treating seeds with colchicine (4). These tetraploids were selfed or crossed with the proper plants to yield the required genotypes. One leaf, of similar size and position on each plant, was selected to be inoculated. Inoculum was prepared by placing a ¼ × ½-in. piece of diseased tissue in 50 ml of nutrient broth, incubating 24 hours at room temperature, and diluting the mixture to 1:2000. The inoculum was applied with a deVilbiss atomizer at 15 lb pressure to water-soak 4 strips, about ½-in. wide, the length of the leaf, 2 on each side of the midrib.

Table 1.—Number of *Pseudomonas tabaci* cells recovered per sample from inoculated leaves of diploid, triploid, and tetraploid plants with 0, 1, 2; 0, 1, 2, 3; and 0, 2, and 4 genes for resistance to wildfire (tests 1 and 2).

	Days after inoculation					
	0	1	2	4	6	8
0/2 [a]	10	50	190,000	800,000	2,900	75
	9	175	295,000	5,120,000	475	250
1/2	10	75	12,000	320,000	300	0
	10	200	7,500	640,000	800	50
2/2	10	25	500	12,800	2,675	125
	12	275	650	4,800	5,500	100
0/3	25	100	20,000	560,000	160,000	17,600
	27	225	75,000	4,640,000	1,666,000	76,800
1/3	18	50	1,250	96,000	5,750	550
	18	175	232,500	144,000	6,750	700
2/3	20	25	1,250	42,000	6,000	475
	22	150	132,500	96,000	9,000	6,000
3/3	30	75	3,250	19,200	7,750	275
	50	200	67,500	32,000	10,000	6,500
0/4	27	25	150,000	3,360,000	144,000	300
	24	150	5,000,000	2,880,000	256,000	225
2/4	15	25	9,000	176,000	66,500	825
	15	150	60,000	128,000	128,000	775
4/4	8	75	750	6,400	75	175
	10	450	2,000	11,200	1,400	140

[a] Gene = numerator; x number = denominator.

The bacterial populations were determined 0, 1, 2, 4, 6, and 8 days after inoculation. The first sample was taken less than 1 hour after inoculation, by cutting 4 disks (one from each inoculated strip) with a 5.5-mm cork borer. Each sample was crushed thoroughly and diluted to 10 ml with sterile distilled water. Serial dilutions were made in sterile distilled water, and 1 ml of each dilution was added to a Petri dish. Melted potato-dextrose agar was then added and thoroughly mixed with the suspension of leaf tissue. The dilution plates were incubated for 48 hours at 25°C, and colony counts then made.

Results and conclusions.—Table 1 shows the population of *Ps. tabaci* in 2x, 3x, and 4x tobacco plants with 0, 1, 2; 0, 1, 2, 3; and 0, 2, and 4 genes for resistance to wildfire. Bacterial populations reached a peak the fourth day after inoculation. On that day the relationship was inverse between the number of genes present for resistance and the population of

Ps. tabaci within the 2x, 3x, and 4x plants; i.e., the population of the bacteria decreased as the genes for resistance increased, and the chromosome number remained constant. The bacterial population was greatest in plants having no genes for resistance, and least in plants having a gene dosage-x relationship of 2/2, 3/3, and 4/4. The population was intermediate in diploid and tetraploid plants with a 1/2 and 2/4 gene dosage-x relationship. Verticillium wilt was found in the triploid plant having the 1/3 gene dosage-x relationship during this experiment, and it is believed that this influenced the rate of multiplication of *Ps. tabaci* in this plant, particularly since the other 3 triploid plants are in the proper position in relation to the diploid and tetraploid plants (Table 1).

It should be noted that symptom expression was as precise an indicator as the dilution plate method since a direct correlation was found between the number of viable bacteria recovered and the symptoms observed. —Kentucky Agricultural Experiment Station, Lexington.

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INOCULATING BARLEY SEEDLINGS WITH USTILAGO NUDA AND WHEAT SEEDLINGS WITH U. TRITICI

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SUMMARY

Grains of barley and wheat were germinated until their coleoptiles were ½–1 cm long; about 2 mm of tissue was cut from the tip of the coleoptiles, the seedlings were placed under partial vacuum in a suspension of chlamydospores of *Ustilago nuda* or *U. tritici*, and the pressure suddenly restored. When seedlings inoculated by this method were planted in autoclaved soil, up to 75% smut-infected heads were obtained in barley and up to 31% in wheat. The time from inoculation to chlamydospore formation was only 6–8 weeks, compared to 6–12 months by floral methods of inoculation.

A number of methods of inoculating barley and wheat artificially with *Ustilago nuda* (Jens.) Rostr. and *Ustilago tritici* (Pers.) Rostr. have been developed since Maddox first showed, in 1895, that he could produce loose smut by dusting the ovary with spores at flowering time (1). Shands and Schaller (4) reviewed these methods, most of which consisted in inoculation of florets with chlamydospores, though a few investigators inoculated barley at host stages other than flowering. Thus, Vandetwalle (7) may have been using *U. nuda* in 1935 when he injected mycelium into the embryos of barley seeds and obtained a low percentage of smutted heads from the inoculated seed. Tapke (6), in 1955, soaked smut-free seed of a susceptible barley variety in water for 24 hours and then split the seed lengthwise with a scalpel. He dipped the exposed embryos into an aqueous suspension of viable spores of *U. nuda* (1 g/L), planted the seed in sterilized soil, and obtained a maximum of 9.4% infection in the resulting plants. In 1959 the writer reported a technique of inoculating wheat and barley with loose smut in the seedling stage (2). A fuller account of this work is given here.

Materials and methods.—The method of inoculation was similar to that used by Rowell and DeVay (3) for the inoculation of seedling corn with *Ustilago maydis* (DC.) Cda. Wheat or barley seed hot-water-treated to free it from the loose smut organism was surface-disinfected with 1% sodium hypochlorite and germinated on water agar in large dishes until the coleoptiles were ½–1 cm long. This was usually accomplished in about 72–96 hours at 20°C. About 2 mm of coleoptile tissue was then cut from the tip of the seedling shoot with a razor blade. The decapitated seedlings were next immersed in a suspension of chlamydospores in sterile distilled water plus 0.02% Tween 20 (polyoxyethylene sorbitan monolaurate). The immersed seedlings were placed in a vacuum at 2–3 in. mercury for 2 minutes, and the pressure was then suddenly restored. The seedlings were sown in autoclaved soil in a greenhouse, oriented with shoots upward before being covered with soil or vermiculite. The concentrations of chlamydospores used ranged from 10.0 to 0.001 g of spores per liter of distilled water.

Results.—Table 1 shows the results of inoculating barley varieties Forrest (C.I. 9187) and Mars (C.I. 7015) with different concentrations of spores. Apart from differences in the highest concentration in the 2 trials (10.0 and 5.0 g/L), the treatments and the general pattern of results were the same; hence the results were combined. The highest concentration of spores gave the highest percentage infection; however, plant emergence was much less than with the 1.0 g/L concentration, which gave an infection percentage almost as high. One-tenth gram per liter gave comparatively low infection, whereas 0.01 g/L gave very little and 0.001 g/L none. Thus, 1.0 g/L was best for routine use. A similar set of inoculations of barley variety Montcalm (C.I. 7149) gave 75% infection at 1.0 g/L. These 3 barley varieties have been infected several times by this method, using chlamydospores at 1.0 g/L.

Table 1.—Emergence and infection obtained[a] when 3- to 4-day-old barley seedlings of 2 varieties were inoculated with *Ustilago nuda* by clipping their coleoptiles and placing the seedlings under vacuum in a chlamydospore suspension in distilled water plus 0.02% Tween 20.

	Barley variety			
	Forrest		Mars	
Spore conc. (g/L)	No. plants emerged[b]	% infection	No. plants emerged[b]	% infection
5.0 or 10.0	17	71	13	61
1.0	34	65	20	60
0.1	36	39	26	38
0.01	32	3	21	9
0.001	38	0	22	0
0.0	35	0	19	0

[a] Combined results of 2 trials with each variety.
[b] Out of 45 seedlings sown.

All the smut-infected heads obtained by this technique were identified as being caused by *U. nuda* by germinating chlamydospores from them (5).

Wheat seedlings have also been infected with *U. tritici* by this method. Spore concentrations ranging from 5.0 to 0.001 g/L resulted in 31% infection of Ceres (C.I. 6900) wheat at the 5 g/L concentration, and 13% infection at 1 g/L (Table 2). Lee (C.I. 12488) and Mida (C.I. 12008) wheat varieties were also infected by this method.

A low percentage of seedling emergence was common, especially with certain treatments. Very high concentrations of spores (5 or 10 g/L) appeared to reduce emergence (Tables 1, 2). Whether this was due to the smut organism itself or to organisms with which the smut collection might have been contaminated, was not determined. Emergence was better in seedlings inoculated with lower concentrations of spores, but even the controls on which no spores were used did not give 100% emergence. This seemed to indicate that either the cutting of the coleoptiles, the placing of the seedlings in a liquid under vacuum, or both could account for the low emergence.

Table 2.—Emergence and infection of 3- to 4-day-old seedlings of Ceres wheat inoculated with *Ustilago tritici* by clipping their coleoptiles and placing the seedlings under vacuum in a chlamydospore suspension in distilled water plus 0.02% Tween 20.

Spore conc. (g/L)	No. plants emerged[a]	% infection
5.0	19	31
1.0	23	13
0.1	21	0
0.01	24	0
0.001	23	0
0.0	23	0

[a] Out of 25 seedlings sown.

Discussion.—Studies of loose smut in greenhouse or field have until now involved the inoculation of florets. Results are erratic because of variations in environmental conditions, especially humidity, and variations in time of flowering between florets in a given spikelet as well as in different spikelets on a given spike. It is difficult also to control the inoculum dosage. By no means the least disadvantage of floral methods is the long period from inoculation to spore production, and the labor involved in field inoculation, tagging, harvesting, and sowing the following season.

The vacuum method of seedling inoculation avoids these disadvantages. Large numbers of seedlings in the same stage of development can be inoculated with the same concentration of chlamydospores under uniform conditions and without danger of contamination with other races of the organism. In addition, smut sori are formed in about 8 weeks, compared to a minimum of several months in the floral method. The method has other advantages: a) Single head collections or races of smut can be increased rapidly and without danger of contamination. b) A small quantity of inoculum is sufficient to infect many plants. c) New wheat and barley lines can be tested for smut reaction in one generation, compared with 2 as required formerly. With this method the plant breeder can determine reaction to the loose smut organisms several months to 1 year earlier than if florets are inoculated. Segregating generations inoculated by this method are automatically selected for resistance since infected plants will not produce seed.

One disadvantage of the method, in a large-scale program, is that the coleoptiles must be cut individually and the seedlings must be planted carefully by hand. Further refinements may speed up these steps and eventually permit the method to be adapted for use in the field. Meanwhile the method is ideally suited to greenhouse investigations of the loose smuts from which it has removed many of the variables inherent in other methods and which it has speeded up from a 6–12 month cycle involving 2 generations of the host to a one host 6–8 week test. A comparison has yet to be made between the infection percentages obtained by the seedling and the floral methods of

inoculation, using both susceptible and resistant barley and wheat varieties.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul.

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TOXIN PRODUCTION BY HELMINTHOSPORIUM VICTORIAE ON SYNTHETIC MEDIA CONTAINING DIFFERENT NITROGEN SOURCES

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SUMMARY

The production of toxin by *Helminthosporium victoriae* was studied on a basic medium containing ammonium nitrate, ammonium sulfate, tryptophan, asparagine, tyrosine, phenylalanine, or methionine as sources of nitrogen. Differences were significant in the amounts of toxin produced by a single-conidium culture of this fungus in 7 media differing in nitrogen sources. A primary toxin was produced that was virulent against Alamo seedlings, and a secondary toxic material that attacked New Nortex seedlings. When Alamo was used for assay, toxin was produced in greatest quantity in media containing ammonium nitrate, followed in descending order by ammonium sulfate, phenylalanine, tryptophan, methionine, tyrosine, and asparagine. The secondary toxin was produced in highest amounts on methionine, followed by phenylalanine, ammonium sulfate, tryptophan, tyrosine, asparagine, and ammonium nitrate.

Introduction.—Meehan and Murphy (4) reported that *Helminthosporium victoriae* Meehan & Murphy produced a toxin that caused symptoms in susceptible oat varieties, and other workers have studied this toxin principle (1, 2, 3, 5, 6, 8). The toxin was partially purified and named victorin (2), and later purified and shown to be polypeptide in nature (5). During the past 5 years, organisms resembling *Helminthosporium sativum* Pam., King, & Bakke have been isolated in Texas from Southland, Bronco, and New Nortex oat varieties. The disease produced by these isolates appeared to be due to a toxin, but less virulent than victorin. Varying degrees of pathogenicity of this fungus have been encountered in a screening program developing resistant varieties of oats. Part of this variability was doubtless due to different genetic factors present in the various isolates and a tendency of the fungus to mutate on artificial media. Preliminary studies with single-conidium cultures indicated that there was variability in pathogenicity due to differences in composition of the media. The present study was made to determine if a single-conidium culture of *H. victoriae* would produce different quantities of toxin when grown on media containing varying nitrogen sources.

Materials and methods.—A single conidium of *H. victoriae* was isolated from Alamo oats and allowed to grow on potato-dextrose agar (PDA). Mass transfers were made to test-tube slants of PDA, and at maximum growth the cultures were stored under sterile mineral oil to reduce mutation and maintain fairly constant characteristics. Transfers from the oil-immersed cultures were grown on PDA plates for subsequent inoculation of liquid media flasks.

Czapek's medium, the basic mineral medium used, was modified by substituting 7 nitrogen-containing compounds for the sodium-nitrate. Compounds were so substituted that the equivalent weight of nitrogen per liter of each medium was constant; thus, compounds having very high molecular weights were used at correspondingly higher rates. Media were prepared and adjusted to pH 6.8; 250 ml of medium were transferred to a 500-ml Erlenmeyer flask and sterilized in the autoclave for 15 minutes at 15 lb pressure. Mass transfers of the actively growing culture of *H. victoriae* were inoculated into the flasks. The cultures were incubated at 27°C and shaken by hand for about 1 minute each day for 30 days. The 30-day-old

cultures were filtered in a Buchner funnel, and the filtrates were adjusted to pH 6.0 and sterilized to kill remaining mycelial fragments and contaminants. The sterile culture filtrates were later used in making dilutions for the seedling assay of toxicity.

Seed of the *H. victoriae*-resistant variety New Nortex and susceptible variety Alamo was allowed to soak 30 minutes in running tap water and removed to a moist chamber to germinate 48–60 hours. Five seedlings with uniform roots about 5 mm long were placed in sterile Petri dishes and irrigated with 5 ml of an appropriate solution according to the method of Luke and Wheeler (2).

Culture filtrates were diluted 1:10 and 1:100 with distilled water and compared to distilled-water checks in tests with both susceptible and resistant varieties. Seedlings were allowed to grow 72 hours at 24–27°C. and the longest root on each seedling was measured. Four dishes of each treatment were included in each test. Three replications of the test were made, and an analysis of variance was made to test statistical significance of differences observed in the experiments.

Results.—The results are in Table 1. When tested on the Alamo variety, media containing inorganic nitrogen compounds produced filtrates containing significantly more toxin material than did the organic sources of nitrogen. The filtrate from the ammonium nitrate medium was most toxic on Alamo seedlings and least toxic on New Nortex seedlings. The ammonium sulfate filtrate ranked second in toxicity on Alamo, and third on New Nortex. Tryptophan in the medium resulted in a filtrate third-most toxic against Alamo and fourth-most toxic on New Nortex. On phenylalanine medium, the filtrate was fourth-most toxic to Alamo and second-most toxic to New Nortex. Methionine in the medium produced a filtrate that was fifth in toxicity to Alamo but first against New Nortex. Culture filtrate produced from medium containing tyrosine ranked sixth in toxicity to Alamo and fifth to New Nortex. Asparagine in the medium caused the least toxicity toward Alamo and ranked next to last in toxicity toward New Nortex.

An analysis of variance of the data showed a significant difference (.05 level of probability) in amount of toxin produced on different media in the test and a highly significant (.01 level of probability) difference among dilutions. The data in Table 1 show that Alamo root elongation was, in every case except two, less than with the corresponding treatment of New Nortex seedlings.

Discussion.—The amount of toxin produced by *H. victoriae* differed significantly on the media tested. Luke and Wheeler (2) reported different rates of toxin production by different single-spore cultures on the same medium. The ability of *H. victoriae* to produce toxin was correlated with both pathogenicity and growth rate.

Data reported in Table 1 showed that amounts of toxin were lowest on media containing the amino acids, and that the most efficient toxin-producing media contained inorganic nitrogen compounds. Culture filtrates produced from ammonium-nitrate- and ammonium-sulfate-containing media were most toxic to the *H. victoriae*-susceptible variety Alamo, and less damaging to the resistant variety New Nortex. In general, the amino acid culture filtrates were less toxic to Alamo and more toxic to New Nortex.

Table 1.—Average root elongation of Alamo and New Nortex oat seed germinating in distilled water[a] and in 2 concentrations of *Helminthosporium victoriae* filtrates produced on culture media containing different sources of nitrogen.

	Root elongation (mm)			
	Alamo		New Nortex	
Source	1:100	1:10	1:100	1:10
Ammonium nitrate	.95	.33	9.21	4.26
DL-phenylalanine	4.22	1.31	4.62	1.69
L-asparagine	7.22	6.14	7.34	6.61
Ammonium sulfate	1.91	.60	5.49	2.08
L-tyrosine	8.46	3.20	7.78	3.53
L-tryptophan	4.52	.83	6.40	1.75
DL-methionine	6.88	2.08	3.12	2.23

[a] Alamo seedlings in distilled water elongated 11.40 mm; New Nortex seedlings in distilled water elongated 11.60 mm.

Luke and Wheeler (2) proposed that *H. victoriae* produces a second toxin that damages resistant as well as susceptible varieties. They named the primary toxin, which was specific for susceptible varieties, victorin. When victorin was purified, it was highly specific for *H. victoriae*-susceptible plants (2, 5, 7). Very weak dilutions produced symptoms that were identical with natural infection symptoms, and resistant varieties were not affected. Pringle and Braun (6) recently isolated from *H. victoriae* a secondary toxin that they called victoxinine. *H. victoriae*-resistant and susceptible varieties are affected equally by victoxinine. Data in this study support the hypothesis that both a primary and secondary toxin are produced. Although no direct evidence is presented to establish the synonymy of the secondary toxins of Luke and Wheeler (2), Pringle and Braun (6), and the presently discussed one, it is likely that they are the same. Toxicity of the amino acid culture filtrates toward New Nortex seedlings may be explained by the presence of the secondary toxin, which can damage all oat plants, as proposed by Luke and Wheeler (2). Production of this type of toxin was favored by the amino acid media, and production of the specific toxin victorin was favored by the inorganic sources of nitrogen.—Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical College System, College Station, Texas, and Crops Research Division, ARS, U.S.D.A.

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THE ROLE OF PECTIC SUBSTANCES IN CHEMICALLY INDUCED RESISTANCE TO FUSARIUM WILT OF TOMATO

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SUMMARY

Tomato plants grown on a calcium-deficient nutrient become more susceptible to Fusarium wilt. Plants treated with the growth regulator naphthalene acetic acid (NAA) become more resistant. Evidence is presented here to suggest that both treatments change the nature of the pectic substances laid down in the plant and that this, in turn affects the resistance of the plant to Fusarium wilt. Analyses have indicated that the stems of calcium-deficient plants contain more water-soluble pectin than normal plants, an increase probably brought on by the lack of calcium bonding of the uronic carboxyl groups in pectin. In contrast, the stems of plants treated with a growth-inhibitive concentration ($10^{-6}M$) of NAA average less water-soluble pectin than normal plants. NAA is believed to inhibit growth by decreasing methoxylation of the uronic carboxyl groups. In consequence the free carboxyl groups in the resulting pectins form calcium salt linkages, which decrease the water-solubility of the pectin. Calcium-deficient plants treated with $10^{-6}M$ NAA contain more water-soluble pectin than normal and are more susceptible to wilt. The pectic substances of calcium deficient tissue were more easily released by pectic enzymes than pectic substances in normal tissue while release was least rapid from NAA treated tissue.

Thus, the nature of the pectic substances in the host is apparently correlated with the resistance of the pectins to hydrolysis by fungal pectolytic enzymes and with resistance of the host to Fusarium wilt.

Introduction.—Previous studies with *Fusarium* wilt of tomato have shown that calcium and the growth regulator a-naphthaleneacetic acid (NAA) interact in their effects on pathogenesis (5). Calcium-deficient plants are more susceptible than normal plants, whereas NAA-treated plants are more resistant. The ability of NAA to induce resistance is nullified by a deficiency of calcium. Thus, the resistance induced by NAA may involve calcium.

These effects probably involve the pectic substances of the tomato plant. First, growth regulators apparently act by altering the nature of the pectins of plants (10). Also, the interaction of calcium and growth regulators would seem to involve these pectins. Finally, one phase of pathogenesis by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans. is concerned with the attack on pectins of the host by hydrolytic enzymes of the fungus.

Recent studies have dealt with the role of growth regulators on the plasticity of cell walls through their action on pectins. Bennet-Clark (1) proposed that growth regulators affect elongation by controlling the proportion of uronic carboxyls to uronic methoxyls in pectic polymers of the cell wall. In a methoxylated state, adjacent pectic chains are attracted by Van der Waals forces, which are weak, and allow wall plasticity and extensibility. If uronic carboxyls predominate, pectic chains are attracted by polar hydrogen bonds. Under these conditions, the pectic substances are less plastic and cell walls are rigid. Glasziou (7) presented evidence that growth regulators influence the binding of pectin methyl esterase to cell walls. If this inactivates the enzyme, which demethylates pectin it would control the proportion of uronic methoxyls to uronic carboxyls.

The interaction of calcium and growth regulators has been observed in physiological investigations. Burstrom (2) and Cooil and Bonner (3) observed that elongation of *Avena* coleoptiles induced by growth regulators can be counteracted by calcium. Tagawa and Bonner (12) found that increased wall plasticity resulting from treatment with indoleacetic acid can be counteracted by calcium.

The observation that calcium counteracts a growth regulator is logically integrated with Bennet-Clark's hypothesis. If uronic carboxyls occur in the presence of divalent calcium, a salt bridge forms, linking adjacent polymers together and thus decreasing plasticity and elongation. If a high proportion of the uronic carboxyls react with calcium, a gel develops. According to this hypothesis, treatment with a growth-stimulatory concentration of growth regulator induces an increase in methoxylated pectic polymers. These are plastic and permit elongation. By contrast, calcium ion counteracts the effect by binding carboxylated pectic polymers through insoluble salt linkages. This

decreases plasticity and inhibits elongation. At inhibitory concentrations of growth regulators there is a decrease in the methoxylation of pectic polymers, which results in an increase of carboxylation and consequent bridging between pectic polymers, by calcium salt formation. When calcium is limiting in the plant, inhibitory amounts of growth regulator cause a shift from the methoxylated to the carboxylated form, but calcium bonding is restricted.

The above hypothesis also explains the results obtained in studies on wilt pathogenesis. *Fusarium* is known to produce pectic enzymes. However, these enzymes cannot attack the calcium bond between pectic polymers. In a calcium-deficient plant, much of its pectic material is subject to hydrolysis. By contrast, in a plant treated with an inhibitive concentration of growth regulator, there is increased calcium bonding and the pectins should be resistant to enzymatic hydrolysis.

The present study examines this hypothesis in terms of how calcium nutrition and treatment with growth regulators may alter the nature of the pectic materials in the tomato stem and the resistance of these pectic substances to enzymatic hydrolysis.

Materials and methods. — *Plant culture.* — Tomato plants were grown to the 4-leaf stage in sand culture. They were then grown for 8 days in Hoagland's solution modified to obtain treatments of low calcium (20 ppm), normal calcium (200 ppm), low calcium plus $10^{-6}M$ NAA, and normal calcium plus $10^{-6}M$ NAA. The NAA treatments were discontinued after 4 days because of good inhibition of elongation, although the calcium regime was maintained for the full 8-day period in all cases. Details of this procedure and the disease indices obtained with these same samples have been reported (5).

Plant analysis.—After the 8-day treatment, stems of one lot of plants were harvested, weighed, dried 3 days at 70°C and cooled, and dry weights were determined.

To obtain the alcohol-insoluble tissue, stems of 30 or more plants for each of the 4 treatments were weighed in fresh condition, sliced into about 5-mm lengths and placed as rapidly as possible in 2 volumes (w/v) of boiling 95% ethanol (70°C). The ethanol was kept boiling for 1 minute and then cooled to room temperature. After 24 hours the ethanol solution was replaced with 63% ethanol. The solution was replaced again after 48 hours. After an additional 72 hours the remaining tissue was air-dried and ground in a Wiley mill with a 20-mesh screen. The calcium content of a sample of this tissue was determined spectrographically.

Two hundred mg of the alcohol-insoluble substance was used for extraction of pectic substances. Two-tenths ml of toluene was added to the samples to retard the growth of microorganisms. Samples were then extracted for two 3-hour periods, each with 40 ml of distilled water on a rotary shaker at 22°C. After each extraction the samples were centrifuged and the supernatant was poured through Pyrex glass-wool filters into a volumetric flask. The small pieces of glass wool in the bases of glass funnels prevented escape of any tissue. These filters were added back to the extracting flask for the subsequent extraction. To the filtrate from the 2 water extractions was added 10 ml of $1M$ KH_2PO_4, adjusted to pH 11 to saponify the methoxy groups, and the volume was made up to 100 ml in the volumetric flask with distilled water. This is the water-soluble pectin fraction.

Following this operation, tissue was subjected to 2 extractions of 30 minutes each at 85°C with 40 ml of 0.4% Calgon (a commercial polyphosphate, Calgon, Inc., Pittsburgh, Pa.), adjusted to pH 6. The extract was separated by centrifuging and filtering, adjusted to pH 11, and brought to 100 ml volume with distilled water. The Calgon extraction was repeated, except that the times of extraction were 3 hours each. Finally, the tissue was extracted twice for 1 hour with 40 ml of boiling $0.013N$ HCl in a reflux condenser. A few ml of water were used to rinse the condensers. The solution was adjusted to pH 11 and brought to 100 ml volume with distilled water. The extracts with Calgon and HCl represent the water-insoluble pectic fractions. The data from 3 experiments were pooled and analyzed statistically.

Calcium-bonded pectate is rendered water-soluble by sequestering the calcium. Although Calgon has frequently been used to remove the calcium, oxalic acid has also been used for this purpose. In these studies 0.5% oxalic acid at 25°C was used instead of Calgon in one experiment.

Purification.—Occasionally, samples of the water-extracted pectin contained impurities that interfered with the color reaction of pectin with carbazole by which uronic acids were determined. To eliminate them, the pectic material was precipitated by adding 2 volumes of acidified 95% ethanol ($0.3N$ HCl) to 10-ml portions of the extract. The precipitate was recovered by filtration and redissolved in 10 ml of warm $0.1M$ phosphate at pH 11.

Uronic acid determination.—The pectic substances were analyzed by the method of McComb and McCready (8), which is based on the reaction of carbazole and uronic acid in 79% sulfuric acid. All samples were analyzed in duplicate, and the uronic acid content of samples was determined colorimetrically with a Coleman colorimeter with 525-mμ filter. Determinations were based on interpolations from a standard curve of the carbazole reaction with known amounts of galacturonic acid, varying from 1 to 40 μg per ml.

Enzymatic hydrolysis.—The proportion of the total pectic substances subject to enzymatic hydrolysis was determined. Forty-mg samples of the alcohol-insoluble substance from each treatment were suspended in 40 ml of $0.02M$ acetate buffer at pH 5.5, containing 0.025% Pectinol 100 D (a commercial pectinase, Rohm & Haas Co., Philadelphia, Pa.). This released the enzyme-hydrolyzable pectic substances. A second sample was placed in buffer containing 0.025% Pectinol 100 D and 0.125% ethylenediaminetetraacetic

acid. This treatment released the total pectic substances, as had been determined by McCready and McComb (9). The differences between the total and the percentage of the total released by enzymes was the unhydrolyzed pectic substance.

The samples were placed on a rotary shaker, and samples were removed periodically to determine the pectic substances released. The samples were filtered through glass wool, adjusted to pH 11 to saponify any methoxyls, and the solutions were brought to a standard volume for analysis by the carbazole method. Since the Pectinol 100 D also contained some carbazole-reactive materials, an appropriate blank containing the enzyme was included to permit correction of the data for this factor.

Experimental results. — Stems of plants receiving low calcium nutrition contained one-third as much calcium as normal stems, whereas plants receiving both low calcium and NAA contained about one-half normal amount of calcium (Table 1). Calcium content tended to increase with NAA treatments. Extraction of stems with alcohol reduced the weight of tissue to about 80% of the initial dry weight. In all treatments the alcohol-insoluble substance contained about 14% pectin. Thus, the several treatments did not affect the total pectic content of tissues. Pectic substances made up about 11% of the dry weight of the stems, and about 0.5% of the fresh weight.

The percentage of pectic substances removed by the various extractions is shown in Table 2. The content of water-soluble pectin was significantly greater in stems of plants receiving low calcium than in plants given normal nutrition and this same trend is shown by plants receiving the low calcium plus NAA treatment. Plants receiving normal calcium plus NAA exhibited the reverse trend, having less water-soluble pectin than normal plants. No significant differences occurred in the percentage of pectic substances released with the first extraction with Calgon. During this period Calgon probably released pectic substances by virtue of its surfactant properties. Distinct differences were noted in the second extraction with Calgon. Tissue grown in the presence of NAA released more pectic substances than normal tissue, whereas calcium-deficient tissue contained less. Stems grown on the combination of calcium deficiency plus NAA released slightly more pectic substances than calcium-deficient stems, but less than normal ones. Yields of pectic material based on the sum of the two Calgon extractions followed the same trend but were not significantly different. Yields of pectic substances released by extraction with dilute acid were quite similar in all treatments.

When oxalic acid was used in the extracting procedure, the percentage of pectic substances released by tissue was similar to the second Calgon extraction and ranked in the same order: low calcium < low calcium, NAA < normal < normal, NAA.

Table 1.—Composition of stems of tomato plants grown on calcium-deficient and normal nutrient solutions, in the presence or in the absence of naphthaleneacetic acid.

Contents of nutrient solution	Dry weight (%)	AIS[a] (%)	Pectic[b] substances (%)	Calcium[b] (%)
Ca-deficient	5.2	3.7	14.0	1.3
Normal	4.6	3.6	13.6	3.2
Normal + NAA[c]	4.9	3.3	14.2	3.3
Ca-deficient + NAA[c]	4.4	3.2	14.6	2.0

[a] Alcohol-insoluble substances (AIS) as % of fresh weight.
[b] % of AIS.
[c] Nutrient solution contained 10^{-6} *M* naphthaleneacetic acid.

Table 2.—The extraction of pectic substances from stems of tomato plants grown in nutrient solutions modified with calcium and naphthaleneacetic acid (NAA).

Extractant	Percent of total pectic substances				
	Low Ca	Normal Ca	Normal Ca+NAA	Low Ca+NAA	LSD 5%
H_2O (1 hour)	14	10	8	12	3
Calgon (1 hour)	34	35	31	33	ns
(6 hours)	36	41	47	39	7
(total)	70	76	78	72	ns
HCl (2 hours)	15	14	13	15	ns

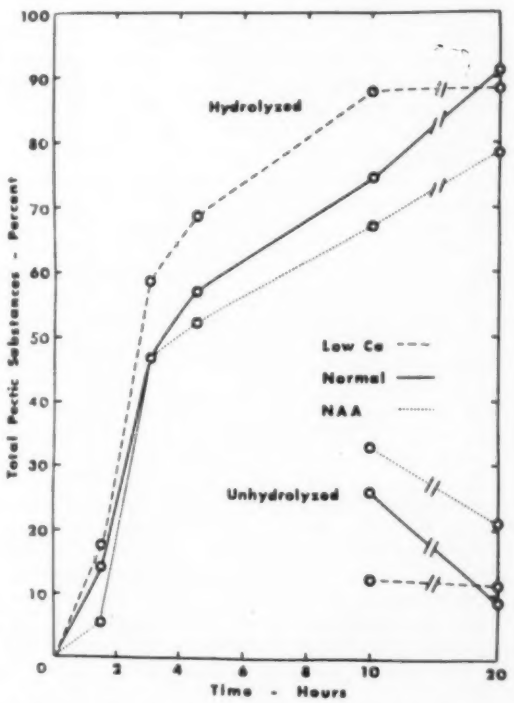

Fig. 1.—The per cent of the total pectic substances enzymatically hydrolyzed in tissue of plants treated with low calcium (–Ca) normal calcium (normal), and napthaleneacetic acid (NAA).

The pectins in the alcohol-insoluble samples from the various treatments were subjected to enzymatic hydrolysis. Two characteristics were of interest: the rate and the extent of hydrolysis. Uronic acid analysis made of reaction aliquots after periods ranging from 1.5 to 20 hours measured the liberation of pectic substances. The percentage of the total released by calcium-deficient tissue was higher than that by normal tissue, except in the 20-hour observation (Fig. 1). The tissue treated with NAA liberated pectic substances more slowly, and hydrolysis did not exceed 80% of the total pectic substances in 20 hours. In 2 more experiments the quantity of pectic substances released was estimated after 20 hours of hydrolysis. The average of the three experiments was respectively 92, 65, and 52% of the total pectic content enzymatically hydrolyzed for low-calcium, normal, and NAA-treated tissue. When data from each experiment were pooled and analyzed statistically, the LSD was 22 at $P = 0.1$.

Discussion.—In previous studies (4, 5, 6), calcium nutrition and treatment with growth regulator were reported to affect wilt resistance. The earlier studies coupled with the present research suggest how such treatments may alter host resistance to the pathogen, viz., by changing the character of the host pectic substances in such a manner as to make them resistant to hydrolysis by the pectic enzymes of the pathogens.

In these studies, a low calcium supply in the nutrient medium decreased resistance to *Fusarium*, decreased resistance to enzymatic hydrolysis, and reduced the amount of calcium per unit of tissue and per unit of pectic substance. Stapp and Hartwich (11) have shown that low calcium in the nutrient supply of potato plants lowers resistance to *Erwinia phytophthora*. The maceration of tissue by bacterial pectic enzymes increased as the total calcium in the tissue per unit of pectic substances decreased. This would indicate that the presence of calcium in tissue interferes with hydrolysis by pectolytic enzymes. The authors have synthesized calcium pectate gels in vitro and found Pectinol 100 D solutions incapable of dissolving the gel. Either the enzyme is incapable of hydrolyzing the calicum-pectate linkage or the calcium released in the hydrolysis is inhibitive to the enzyme. Thus McCready and McComb (9) obtained similar yields of pectic substances, using the combination of ethylene-diaminetetraacetic acid and Pectinol 100 D, as compared with other standard methods. Pectinol 100 D required only the removal of metals by the chelating agent to permit complete hydrolysis.

In these studies NAA caused only a slight increase in total calcium uptake. Pectic-bound calcium, however, increased more than the calcium uptake did, as measured by the quantity of pectic substances released by either Calgon or oxalic acid. An increase in pectic-bound calcium would be reflected as a decrease in water-soluble pectin. Water solubility could also be influenced by pectic chain length. This same effect was observed by Mulder (10), who studied the effects of a growth inhibitory concentration of indoleacetic acid on the amount of water-soluble pectin in *Avena* roots. Mulder found no effect of the growth regulator on celluloses, hemicellulose, or protein solubility. Although the effect observed by Mulder was greater than in the present study, both are in the same direction. Differences in the magnitude of the effect can be accounted for in the differences in tissue under study and in the analytical methods used in determining water-soluble pectin.

Thus, it appears that growth regulators may confer wilt resistance by inducing calcium bonding, which results in inhibition of elongation of growing cells. The use of growth regulators as a means of disease control will, of course, be limited to cases where the inhibition of growth is not important in crop production.—Department of Plant Pathology and Botany, Connecticut Agricultural Experiment Station, New Haven.

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ROD-SHAPED PARTICLES ASSOCIATED WITH VIROSIS OF SPRUCE

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Part of C. Sc. thesis by the first author, Biological Institute of Czechoslovak Academy of Sciences, Prague.
The authors are indebted to Miss H. Branišová and J. Šafr for technical assistance, and to Dr. A. Pintera, who supplied the aphid infected seedling trees at our disposal.

SUMMARY

Rod-shaped particles were found in the de-resined exudates of woody twigs of virus-diseased spruce trees (*Picea excelsa*). They are present in the naturally infected trees and in spruce seedlings inoculated by aphid transmission (*Sacchiphantes abietis*) or by grafting; no similar structures were found in healthy control plants. High-resolution microscopy revealed the hexagonal structure of these rods, 49±3 mμ in diameter. The distribution curve of particle length is rather broad (200–3000 mμ), with the mean at 625 mμ.

Introduction.—The etiology of a discoloration and deformation of conifers has not yet been explained. Tubeuf (9) was unable to isolate a pathogen, and included these phenomena under physiological disorders without any further experimental proof. Blattný (1, 2) described a disease of spruce trees from South Bohemia, and inferred that it might be of virus origin. Shortly after, Smolák (8) reported a similar observation from Eastern Bohemia, and called the aphid *Sacchiphantes abietis* L. (*Chermes abietis* L.) a possible vector. In preliminary notes on long-term experiments with the spruce virosis Pintera (7) reported aphid transmission of a yellowing of spruce needles. Blattný (3) reported positive results from graft experiments.

The symptoms of spruce virosis vary with season, tree age, and length of infection (Fig. 1). The early stages usually exhibit yellowish-green or yellowish-white foliage on some twigs or on the whole trees. They are most striking in September and October, and disappear in spring. In later stages the chlorotic symptoms are sometimes not visible and the trees manifest some growth disorders (shortened needles on shoots, asymmetrical development of twigs, loss of the top). In the fall some of the needles get dry, and fall. A detailed description of the disease will be published later.

To speed studies of this disease (the first symptoms occur one or even more years after infection) an attempt was made to detect the specific cytoplasmic particles with electron microscopy.

Materials and methods.—Naturally infected spruce trees (*Picea excelsa* L.) about 60 years old were used in the first series of experiments. They were in South Bohemia and had been under observation for many years. Control healthy material was collected in mountain localities where symptoms had not been observed. Needles were ground in buffer and distilled water (1:1:7 w/v/v). The buffer was 63 ml of 0.005*M* sodium cacodylate, 14 ml 1*N* NaOH, 20 ml 4*M* K_2SO_3, and 3 ml nicotine. Expressed sap (pH 6.8–7.5) was centrifuged 10 minutes at 10,000 G. Clear yellow supernatant, after serial dilution with water (1:4), was dropped on a collodion membrane floated on water. The drops were dialyzed directly on it and after lowering of the water surface the mounts on the membrane were situated and set on grids laid under it (6). The specimens were then dried and shadow-cast with palladium.

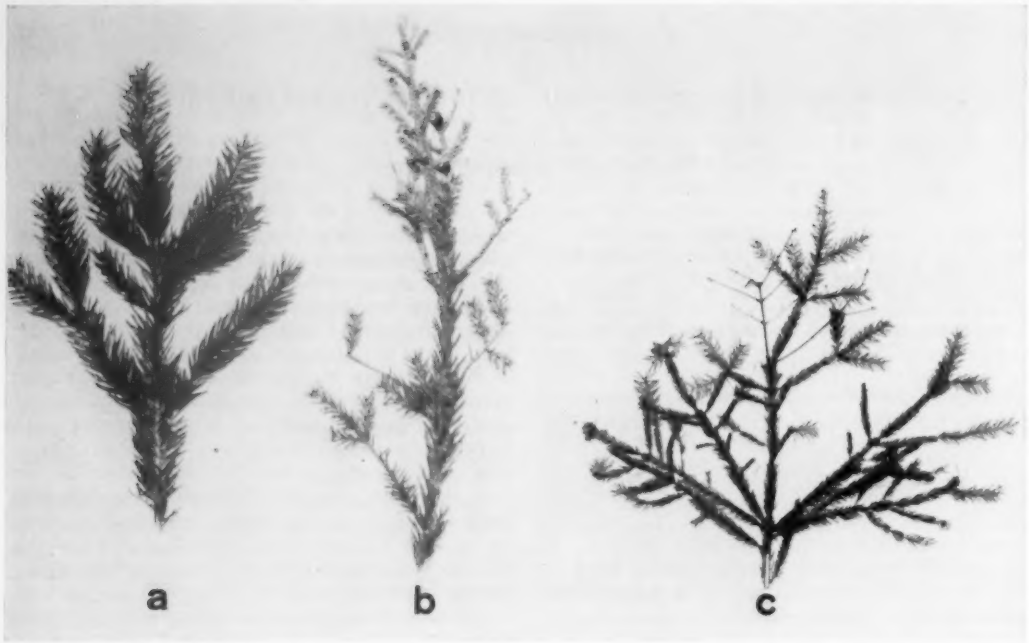
The second experimental series was performed on spruce seedlings infected by *Sacchiphantes abietis* or by grafts 1–3 years earlier. The source of infection was the plants from the first experiments. The control healthy seedlings came from high mountain areas where neither symptoms nor aphids had been found during long observation. Unbranched twigs (2–5 mm in diameter) were attached in Johnson's exudate apparatus (5). The top woody part was cut off, and water pressure of 4.0–5.5 atm was applied to the lower end of the twig. Exudate drops were then mounted on the collodion-coated electron microscope grids and allowed to dry. However, a resinous and lipidlike coating on such specimens, originating from the expressed fluid, made observation of the structures impossible. The grids were therefore put on a sheet of thick filter paper and thoroughly washed with about 100 drops of chloroform before being shadow-cast with platinum and carbon.

The particle length was examined with a Tesla BS 242 table electron microscope. Two lots of polystyrene latex particles (188 and 1171 mμ) served as size standards. Photographs of selected specimen grids were taken with a Tesla 413 electron microscope at ×22,500; measurements were made at ×250,000.

Results.—In the first series of preparations from diseased spruce trees, aggregates of rod-shaped particles were observed as shown in Fig. 2; they were covered with cytoplasm. This homogenate-extract method was very laborious and succeeded in only about 20% of the experiments.

Johnson's exudate method, in the second series, was convenient. Fifteen infected spruce seedlings were examined with this method, six specimen grids prepared independently from each. Rod-shaped particles were observed in all mounts (Fig. 3), whereas no similar structures were found in 9 healthy seedlings examined, 6–10 independent specimens made from each.

Occasionally, distinct bundles of flexuous fibers could be seen in preparations from both infected and healthy trees, but these were not transparent to the electron beam, as were the specific rods. Similar fibers, however, sometimes occur in exudates from



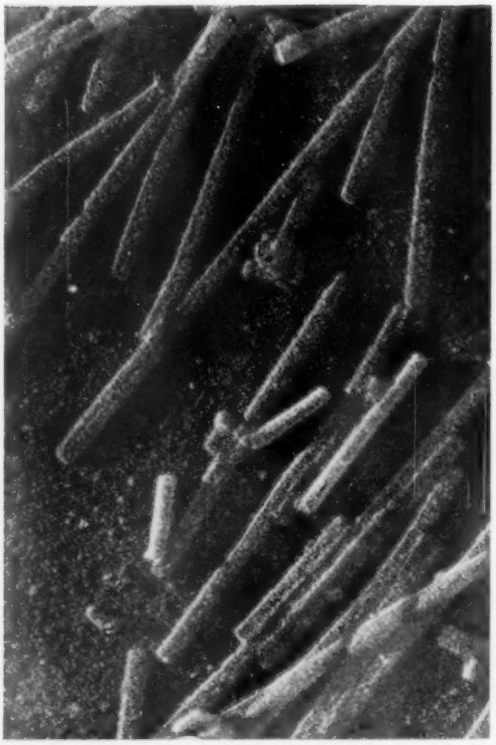


Fig. 1–4.—**1)** Healthy and diseased spruce. **a)** Healthy spruce twig. **b)** Asymmetrical growth, loss of needles associated with the spruce virosis. **c)** Asymmetrical growth, needles alternately reduced and normal in length. **2)** Electron micrograph of needle homogenate from naturally infected spruce trees. Shadowed with palladium (×30,000). **3)** Rodlike particles in exudates from spruce seedlings infected by aphids obtained by Johnson's procedure from woody twigs. Platinum shadowed (×70,500). **4)** Highly enlarged rods showing the hexagonal structure. Platinum shadowed (×114,000).

herbaceous plants, too. They are probably freed cellulose fibers, since both the membrane and the specific rodlike particles were broken after prolonged irradiation in the microscope in a few cases. The artifact fibers, in contrast, remained and even bridged the broken membrane over.

The length of 2370 particles was measured from the exudate preparations. The distribution diagram of lengths, however, shows a rather unusual variability, with the most frequent length around 625 mμ (Fig. 5). High-solution pictures revealed the hexagonal shape of these rods. The diameter, 49±3 mμ, was determined on the particles that lay with their long axis in the direction of shadowing. The width of the hexagon walls (about 26 mμ) corresponds with the geometry of these particles (Fig. 3, 4).

Discussion.—In the first experiments it was recognized that interfering factors in spruce trees make simple plant extracts or saps unsuitable for such study. These factors are: a relative high acidity, tannins, and polyphenolic substances. Even if most were eliminated from the extract solution, it appears that these methods would not be very reliable. Some cell constituents would still be adsorbed on the particles studied.

The second series of experiments with the exudates also had to show the transmissibility of specific particles, as well as symptoms of spruce virosis, by aphids and grafts. The exudate mounts, followed by washing the specimens with chloroform, are more reliable than methods used before. Rodlike particles were found not only in the woody parts of branches with pronounced symptoms but also in nearly symptomless branches from all sections of the tree (top, central, or bottom). It was difficult to get exudates of detached needles. In such specimens particles occur only seldom. All this is apparently connected with the morphology of coniferous foliage.

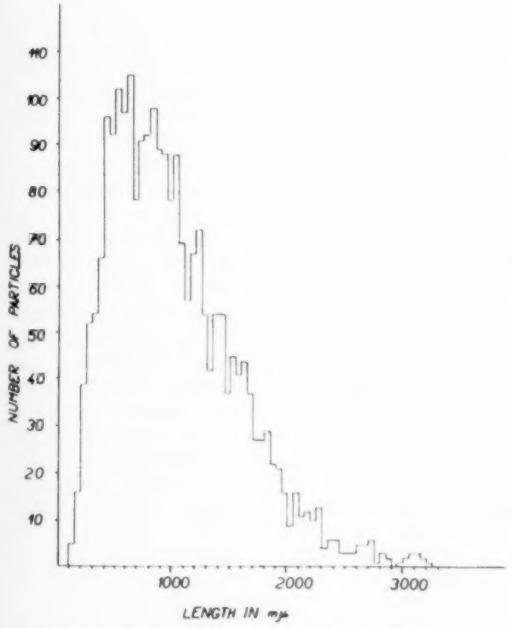


Fig. 5.—Length distribution of viruslike particles in exudates from spruce.

The distribution curve of particle length is unusually broad, contrary to present findings for other rorlike viruses, e.g., Brandes and Wetter (4). In spite of the nearly neutral pH of exudates (about pH 6.2) the possibility of end-to-end aggregation of rods cannot be excluded, because it is striking that the secondary maxima in the diagram (Fig. 5), lying about the main maximum of 625 mμ, repeat in nearly regular distances of about 200 mμ.

The cleaning effect of chloroform treatment of specimen grids before shadowing is most surprising. In addition it allowed direct observations of the fine structure of rods. The hexagonal structure, together with the fact that these particles occur in diseased spruce only, strongly supports the idea that it is a virus particle.—Department of Plant Pathology, Biological Institute of Czechoslovak Academy of Sciences, Prague 6, Na Karlovce 1, Czechoslovakia.

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BOOK LISTING

THE ECOLOGY OF SOIL FUNGI. An international symposium edited by D. Parkinson and J. S. Waid. Liverpool University Press, 123 Grove Street, Liverpool 7, England.

The activities and interrelationships of fungi in soil provided the basis for a symposium held at the University of Liverpool during August 19-21, 1958. The book consists of 6 major parts. Each part includes a review of 1 aspect of the subject, 3-5 shorter papers, and a lively discussion at the end. he 6 major parts are: methods for isolation and estimation of activity of fungi in soil; the growth of fungi in soil; antagonisms in soil; dynamic equilibria of soil populations; problems associated with the decomposition of organic matter in soils; physiology of soil fungi.

ARTIFICIAL INOCULATION OF LONGLEAF PINE WITH SCIRRHIA ACICOLA

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Accepted for publication September 12, 1960.

SUMMARY

Longleaf pine was infected with *Scirrhia acicola* isolated from loblolly as well as longleaf pine trees. About 8–11 weeks were required for expression of symptoms. Infection occurred only on immature longleaf needles 3–6 in. long, and not on loblolly pine. Cultural characters varied widely from isolate to isolate, and were instable following successive transfer. No correlation was found between cultural differences and percentage of infection, types of symptoms, or time of symptom expression on longleaf pine. Isolates from longleaf were, in general, more infective than those from loblolly.

Scirrhia acicola (Dearn.) Siggers, long known as the most serious pathogen of longleaf pine (*Pinus palustris* Mill.), was reported in 1952 to cause a prevalent needle dieback of loblolly pine (*P. taeda* L.) in the Carolinas (1). More recently, severe browning of loblolly pine has been observed as far west as Texas and Arkansas.

Boyce (1) inoculated loblolly and slash pine (*Pinus elliottii* var. *elliottii*, Little and Dorman) and infected the latter with *S. acicola* isolated from the former. Cross infection has not been reported between longleaf and loblolly pine. Siggers (5) and Hedgcock (2) infected longleaf and and slash pine, but experienced difficulty in producing infection with conidial spore suspensions.

Snow (6) found considerable variation among isolates from longleaf and loblolly pine, and the differences were not correlated with host species.

The 6 experiments reported here were undertaken to: (a) develop a satisfactory method of infecting pine seedlings with *S. acicola*, (b) determine the incubation period, (c) attempt to cross-infect longleaf and loblolly pine with isolates from opposite hosts, and (d) compare cultural differences with pathogenicity.

Materials and methods.—During 1958, loblolly and longleaf pine seedlings were inoculated with single-spore cultures of *S. acicola* from longleaf and loblolly pines in different parts of Harrison County, Mississippi. The seedlings were 2 years old and had been grown in pots. They were sprayed with bordeaux mixture to keep natural infection to a minimum.

In experiments 1 and 2 the plants had fully developed needles that had been sprayed with the fungicide. This foliage was washed with soap and water before inoculation. In succeeding experiments, all the old needles were removed and only the new unsprayed needles were inoculated.

Conidial suspensions, prepared in sterile distilled water from three-week-old cultures on malt agar, were adjusted to about 250,000 conidia per ml and sprayed on the plants with a hand atomizer. The inoculum was also sprayed on glycerine-coated glass slides, which were placed under the plants and later observed for spore germination. Control plants were sprayed with sterile distilled water.

Immediately following inoculation, plants were placed in humidity chambers for 128 hours. A polyethylene tent was used in experiments 1 and 2, whereas in experiments 3 and 4 each plant was covered with a polyethylene bag. A wet canvas tent, as described by Jewell (3), was constructed for experiments 5 and 6.

The canvas tent maintained moderate temperatures and high relative humidity, and it exposed all the plants in one experiment to about the same condition. Some needles bent at the leaf sheath during the extended dark period, but this could probably be overcome by adding windows to the tent and covering the plants for a shorter time. Needle scalding on some plants under polyethylene bags masked early brown-spot symptoms in experiment 4.

Cultural differences on malt agar and potato-dextrose agar were determined for one-month-old cultures of 6 of the original single-spore isolates. Later an additional 120 different isolates from longleaf trees were examined for cultural characteristics on malt agar slants.

Results.—In all tests 65–90% of the conidia on glass slides germinated. Leaf impressions (4) taken after the plants were removed from the humidity chambers showed conidial germination on both longleaf and loblolly needles in all experiments. Fig. 1 shows a leaf impression with a germinated conidium.

The dates when symptoms were first seen on longleaf are listed in Table 1. Light greenish-gray spots were followed by the characteristic brown spots, needle browning, and Pycnidia formation. The symptoms were similar on all plants. Siggers (5) found some lesions on longleaf 2 weeks after inoculation, whereas Hedgcock (2) reported that 6–8 weeks elapsed before the first symptoms occurred. The present tests suggest that an even longer incubation period (8–11 weeks) is common. The age of longleaf needles apparently is a factor in susceptibility: infection occurred only on immature needles 3–6 in. long. With loblolly, Boyce (1) found that mature needles were more susceptible than young needles and developed symptoms sooner. The differences in the age at which needles are susceptible may explain why symptoms

Table 1.—Inoculation of longleaf pine with *Scirrhia acicola*..

Experiment no.	Inoculation date	No. of trees	Needle length (in.)	Isolate	Source of isolate	Incubation period (weeks)	% needles with symptoms
1	December 19	8	12–14	2	longleaf		1
		2	12–14	control			1
2	January 15	3	12–14	3	longleaf		1
		2	12–14	control			1
3	February 28	3	3–5	1	longleaf	11	54, 89, 92[a]
		3	3–5	22	loblolly	11	47, 83, 88[a]
		2	3–5	control			0, 0
4	April 1	2	4–6	1	longleaf	15[b]	43, 75
		2	4–6	2	longleaf	15[b]	60, 75[a]
		2	4–6	3	longleaf	15[b]	12, 93
		2	4–6	20	loblolly	15[b]	1, 6
		2	4–6	21	loblolly	15[b]	10, 22
		2	4–6	22	loblolly	15[b]	42, 56[a]
		2	4–6	control		15[b]	3, 4
5	May 9	2	4–6	1	longleaf	10	11, 60[a]
		2	4–6	2	longleaf	11	12, 26[a]
		2	4–6	3	longleaf	8	42, 90[a]
		2	4–6	20	loblolly	11	1, 8[a]
		2	4–6	21	loblolly	11	2, 4[a]
		2	4–6	22	loblolly	11	11, 19[a]
		2	4–6	control		11	7, 8
6	August 5	2	12–16	5	longleaf		3
		2	12–16	6	longleaf		3
		2	12–16	25	loblolly		3
		2	12–16	26	loblolly		3
		2	12–16	control			3

[a] *S. acicola* reisolated from inoculated plants.
[b] Early symptoms masked by scalding of needles in plastic bags.

usually appear on longleaf in spring, and on loblolly in late summer and early fall (1).

Several *S. acicola* isolates from loblolly pine infected longleaf. Except for the percentage of infected needles, no differences could be seen in the effects of longleaf and loblolly isolates.

None of the trials, even those with inoculum from the same host, developed brown spot on loblolly pine. The age and condition of loblolly needles were approximately the same as those of longleaf in each experiment. The December and January inoculations of tests 1 and 2 were on mature needles sprayed with bordeaux mixture. In experiments 3–6, only unsprayed needles were inoculated, and they ranged from 1–2 in. long in February to full size in August. All loblolly seedlings were held until December, 1958, before they were discarded.

All 6 original isolates were black on potato-dextrose agar; one isolate, 21, sectored frequently on this medium. On malt agar, 3 of the 6 cultures were black, 1 was white, and 1 gray. All except one sporulated abundantly on both media.

Cultural differences could not be correlated with percentage of infection, types of symptoms, or time of symptom expression on longleaf pine (Table 1). For example, isolate 3 from longleaf caused severe infection in experiments 4 and 5, whereas isolate 20 produced little or no infection in either experiment. The cultural characters of these two isolates were the same in all respects.

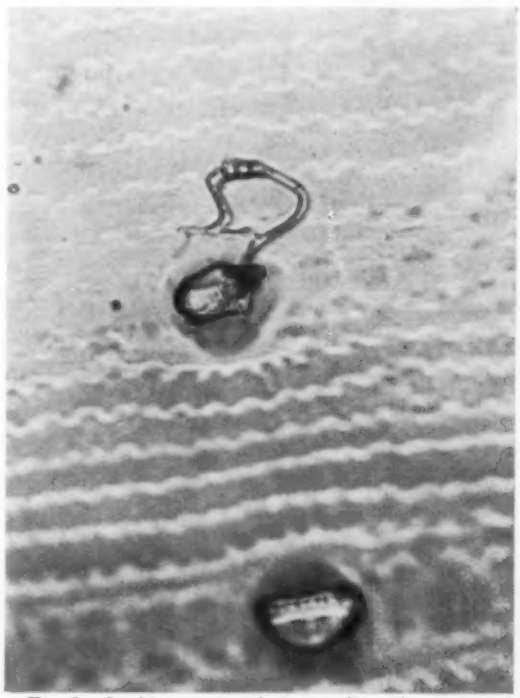

Fig. 1.—Leaf impression of pine needle with germinated spore and hypha of *Scirrhia acicola* entering a stomatal cavity.

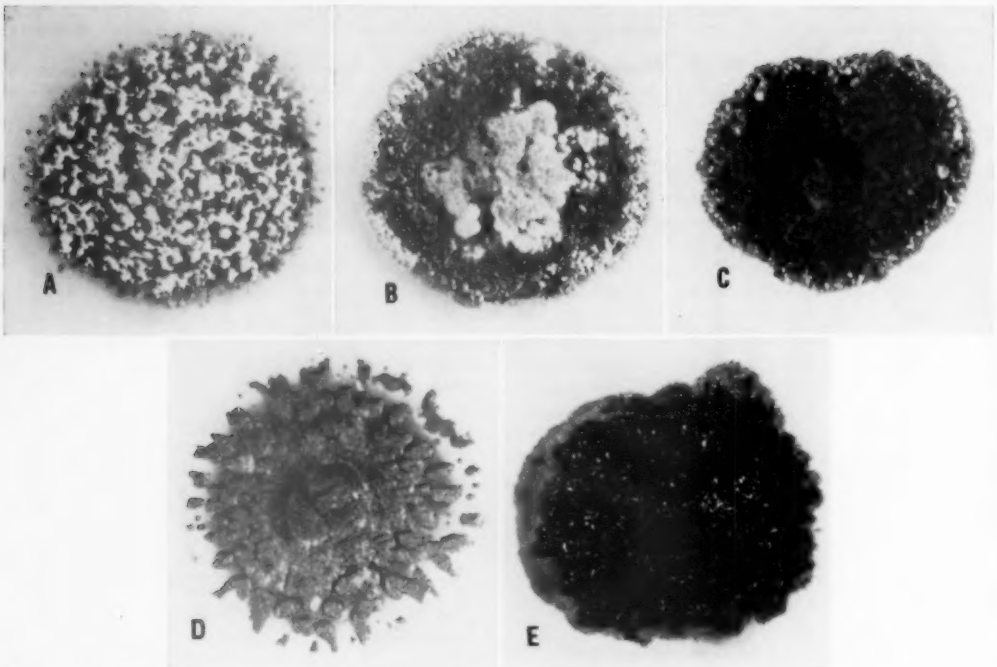

Fig. 2.—Variation in *Scirrhia acicola* in culture. A, B, and C on 2% malt agar; D with, and E without, sectoring on PDA.

One type of sectoring and differences in color of cultures is illustrated in Fig. 2. The characters were unstable, and their original appearances changed after several transfers.

The 120 isolates later observed represented all gradations in color from black to white, but the majority were various shades of gray. The light colors (gray and white) were caused by aerial mycelium, the dark colors by masses of conidia in a dark gelatinous matrix.

There was no evidence that variations in cultural characters indicate differences in pathogenicity of the organism in either pine host. In general, the isolates from loblolly were less pathogenic than those from longleaf.

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BOOK LISTING

NEMATOLOGY FUNDAMENTALS AND RECENT ADVANCES WITH EMPHASIS ON PLANT PARASITIC AND SOIL FORMS. Ed. J. N. Sasser and W. R. Jenkins. University of North Carolina Press, Chapel Hill. 480 p. $12.50.

This advanced text and reference volume is the outgrowth of a series of lectures and laboratory exercises presented in 1959 during a Southern Regional Graduate Summer Session in Nematology at North Carolina State College. Included are methods, morphology and anatomy, physiology, genetics and cytology, ecology, host-parasite relations, and control. A National Science Foundation grant enabled summer session participation by authorities from various parts of North America and Europe. Twenty-two of these each contributed 1 or more of the 47 chapters. Extensive literature citations are provided for each chapter, and the book is well illustrated.

TEMPERATURE IN RELATION TO LOOSE SMUT IN BARLEY AND WHEAT

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SUMMARY

When wheat and barley plants infected with loose smut were grown from seeding to spike emergence at each of 3 temepratures, 65, 75, and 85°F, "normal" smutted spikes were produced at 65°F; at 75°F both "normal" smutted spikes and spikes with reduced chlamydospore formation were obtained; at 85°F slender green heads with few to no chlamydospores were formed. Seven days at 85°F between the beginning of internode elongation and the boot stages almost completely prevented chlamydospore formation in smut-infected plants. Smut infection of leaves was common in the greenhouse in both cereals, but at 85°F smut-infected barley leaves had yellow chlorotic streaks instead of the black elongated smut sori formed at lower temperatures. Varieties differed in susceptibility to leaf infection and in degree of inhibition of chlamydospore and sorus formation produced in infected spikes by specific periods at high temperatures.

Introduction.—A number of investigations are recorded in which the effect of temperature at sowing time was studied in relation to subsequent infection of wheat or barley with loose smut (1, 2, 5, 8, 11). The writer was the first to record the effect of the temperature following planting on loose smut development and expression (3). Presented here is a fuller account of this work, together with later results.

Materials and methods.—Three varieties of wheat—Ceres (C.I. 6900), Lee (C.I. 12488), Marquis (C.I. 3641)—and 6 varieties of barley—Forrest (C.I. 9187), Kindred (C.I. 6969), Mars (C.I. 7015), Montcalm (C.I. 7149), Parkland (C.I. 10001), and Traill (C.I. 9538)—were respectively florally inoculated in the field with *Ustilago tritici* (Pers.) Rostr. and *Ustilago nuda* (Jens.) Rostr. by the Moore and Munnecke (7) air-blast method. Uninoculated spikes from the same seed lots were harvested to use as controls. The inoculum used was collected at random from field plots at St. Paul.

The inoculated seed was grown in a preliminary test in the greenhouse to determine the level of smut infection. In Ceres and Lee wheat and in Forrest, Montcalm, and Traill barley varieties the percentage of infected spikes was 60 or over. In Marquis wheat the percentage infection was about 48. Mars, Parkland, and Kindred barley varieties each had about 20% infection with loose smut. Four sets of experiments were performed:

1) Smut-infected and smut-free wheat and barley seeds were planted and the plants obtained grown to spike emergence in greenhouses set at 65, 75, and 85°F. 2) Smut-infected and smut-free seeds of both cereals were planted in the 65°F and 85°F greenhouses, and the plants interchanged from one temperature to the other at different stages of host development from planting to spike emergence. 3) Smut-infected and smut-free seeds of both cereals were planted at 65°F, the plants obtained were transferred to 85°F at different stages of host development from planting to spike emergence, and transferred back after 5–7 days. 4) Smut-infected and smut-free wheat and barley plants were grown from seeding to spike emergence at 85°F during the day and 65°F at night, and vice versa.

The seeds, treated with a Ceresan-Arasan mixture, were planted 6 per pot in 4-in. pots of autoclaved soil, and the plants thinned to 5 per pot. Four pots per treatment were usually employed for each variety. The pots were randomly arranged on the benches, and randomly rearranged at intervals to eliminate position effects.

The greenhouses were thermostatically set at 65, 75, and 85°F. Thermograph records showed that night temperatures were constant and day temperatures fluctuated no more than about 5°F, averaging about 65, 75, and 85°F over each 24-hour period. Supplemental light was provided each evening and early morning and throughout cloudy days by 300-W incandescent bulbs 3¼ ft above the plants.

Results. — When loose-smut-infected and smut-free seeds of wheat and barley varieties were planted and the plants obtained grown to spike emergence at each of the 3 temperatures, normal smutted and smut-free spikes developed at continuous 65°F. At continuous 75°F many normally smutted spikes were produced by both cereals, but reduced sorus and chlamydospore formation was also common. At 75°F, wheat developed all gradations from normally smutted spikes to spikes that looked smut-free but had a few tiny sori dotted along the glumes (Fig. 1, 2). The florets of such spikes often contained smut sori instead of ovaries. Barley also had a range of symptoms from spikes with normal chlamydospore development to spikes with a sterile rachis and no chlamydospores (Fig. 3). At continuous 75°F there appeared to be varietal differences in symptoms in both cereals.

At 85°F the spikes were normal on smut-free plants of both cereals but on smut-infected plants were greenish and quite unlike the dark-brown to black smut-infected spikes produced at lower temperatures. Infected wheat spikes at 85°F were slender and

Fig. 1–2.—**1)** Kinds of smut symptoms in Ceres wheat grown throughout at 75°F; all 4 spikes are infected. **2)** Spikelets from the spike second from the right in Fig. 1 showing tiny sori on glumes and floral parts.

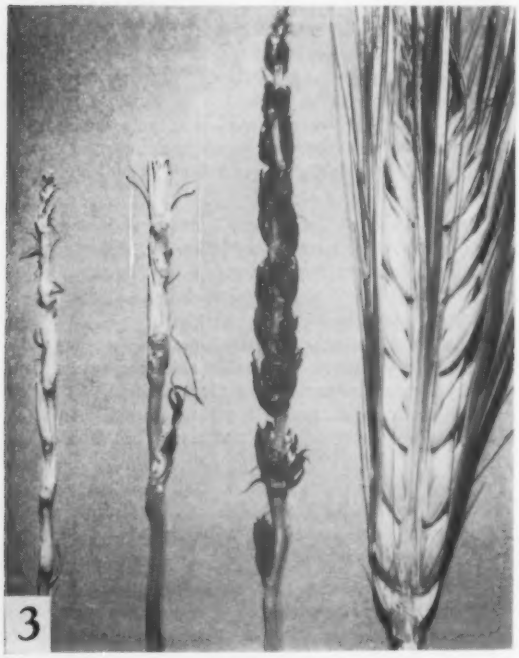

Fig. 3.—Spikes of Montcalm barley from plants grown throughout at 75°F; smut-free on right, second from right normal smutted spike; next two show degrees of reduced smutting—that on the left has no chlamydospores.

unevenly light green. Glumes, and often lemmas and paleae, were usually formed on these spikes, but stamens and pistils were seldom present (Fig. 4). Tiny sori frequently dotted the glumes and awns, but on other plants external evidence of chlamydospore formation was usually lacking. The latter spikes were identified as smut-infected both by clearing and staining them by an adaptation of an embryo technique for loose smut identification (4) and by isolating the fungus from them by plating pieces of surface-disinfected spikes on potato-dextrose agar (PDA).

The spikes of infected barley plants at 85°F were very slender and light-green to creamy-white. Some smut-infected spikes had no external evidence of chlamydospores, but stamens and pistils were usually absent (Fig. 5). Presence of the fungus was established as with wheat. Other smut-infected barley spikes at 85°F had a few green to light-brown sori replacing groups of spikelets on the rachis.

Temperature had little effect on loose smut development until the internodes had begun to elongate. Thereafter, the longer that plants were grown at 85°F the more pronounced was the reduction in sorus and chlamydospore formation. Seven days at 85°F almost completely inhibited chlamydospore formation at the stage between early internode elongation and the boot stages of development. In wheat this effect could be produced by placing the plants at 85°F as late as the last 7 days before spike emergence. For comparable results with barley, the 7th day at 85°F should coincide with the 2nd or 3rd day before spike

Fig. 4–5.—4) One smut-free spike (left) and 3 smut-infected spikes of Ceres wheat grown throughout at 85°F; no sori visible externally on smut-infected spikes. **5)** One smut-free (right) and 3 smut-infected spikes of Traill barley from plants grown throughout at 85°F; the spike second from right has a few tiny brown sori, the other 2 smut-infected spikes have no visible sori or chlamydospores.

emergence. With both cereals as little as 5 days at the 85°F temperature caused a pronounced reduction in chlamydospore formation in spikes of infected plants.

To determine whether this temperature effect might take place in the field, an experiment was set up to simulate natural diurnal fluctuations in temperature. Loose-smut-infected and unifected Lee and Ceres wheats and Montcalm and Traill barley varieties were moved at 9 A.M. and 5 A.M. each day during the 6-week growing period (seeding to spike emergence) so that they were at 85°F for 8 hours each day and at 65°F for 16 hours each night, and vice versa.

Eight hours at 85°F caused slight reduction in chlamydospore formation in smut-infected wheat heads, but 16 hours at 85°F each night caused a marked reduction in Ceres but little in Lee.

Eight hours per day at 85°F had no discernible effect on loose-smut-infected barley plants. Sixteen hours at 85°F each night caused a slight reduction in chlamydospore formation in Traill but none in Montcalm. It is probable that the effect of the 16 hours at 85°F each night on the smut-infected wheat and barley plants was directly caused by the high temperature rather than by the reversal of the normal temperature-light conditions in the field.

Infection of wheat and barley leaves with loose smut was frequent in the greenhouse. The amount of infection and its expression were both influenced by certain temperatures. In wheat, leaf infection was frequent in plants grown at 65 but not found at 75 or 85°F. In barley, however, infection was much more pronounced at 75 than 65°F, though it was frequent

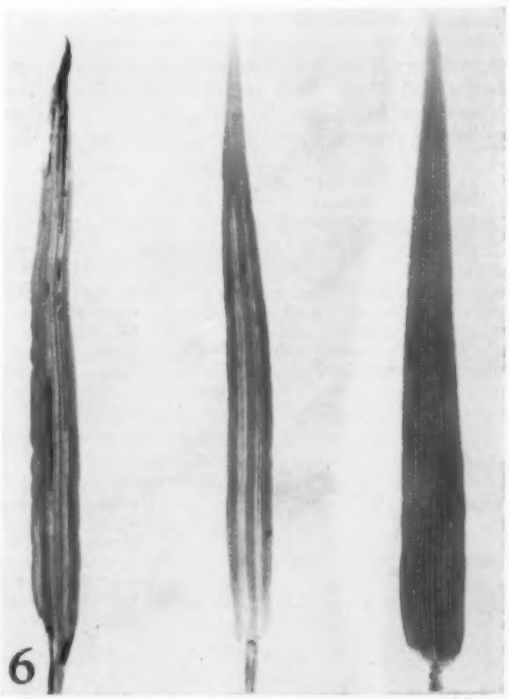

Fig. 6.—Montcalm barley leaves: smut-free (right); smut-infected (left) grown throughout at 75°F has chlorotic striping plus abundant chlamydospore formation; smut-infected (center) grown throughout at 85°F has chlorotic striping only.

at the lower temperature. At 85°F barley leaves infected with smut had long chlorotic streaks similar to early symptoms of barley false stripe virus (Fig. 6). The fungus was isolated from these chlorotic areas by plating pieces of washed leaves on PDA. When smut-infected barley plants were transferred from 85 to 65°F at different stages of host development, more black and less chlorotic streaking developed the longer the plants were at the lower temperature, and vice versa. In both cereals varietal differences were found in susceptibility to leaf infection.

The reactions of smut-infected wheat and barley varieties to temperature are summarized in Table 1.

So that reaction of the organism to temperature could be compared with growth of the healthy host, a monochlamydospore culture of *U. nuda* was grown at 6 temperatures—50, 59, 68, 77, 86, and 95°F—for 4 weeks on an enriched potato-sucrose agar (400 g potatoes, 100 g sucrose, 1 g asparagine, 2.5 g yeast extract, 17 g agar per L). For the first 5 days the cultures were held at room temperature to allow growth to begin. Then twelve 125-ml Erlenmeyer flasks, each containing 25 ml of medium, were placed at each of the 6 temperatures. The temperatures at which mycelium grew well in culture were those at which chyamydospores were produced in loose-smut-infected barley plants in the greenhouse, i.e., 68 and 77°F (Table 2). Observations and measurements made on smut-infected and smut-free plants of the 2 hosts at the 3 temperatures used show that the 65°F temperature is the most favourable for growth of both barley and wheat. Both smut-infected and smut-free plants of all the wheat and barley varieties used were taller and the spikes were longer at 65 than at either 75 or 85°F. The 85°F temperature was unfavourable for both cereals, but especially barley; at that temperature the spikes on the primary culms did not always emerge.

Discussion.—The study has shown for the first time that temperature has an important influence on chlamydospore development, and consequently on symptom expression in the loose smuts.

The separate reactions to temperature of the hosts and parasites under study lead to the following conclusions:

1) Development of the hosts is excellent at temperatures that favour growth of the smut organisms.

2) The high temperature levels tolerated by the disease organisms are similar to those tolerated by the hosts.

3) The 85°F temperature is slightly less favorable for the development of chlamydospores than for the diseased plants, since the latter appear smut-free with even short exposures to the high temperature. The fungus is present, however, in the spikes of diseased plants at the high temperature even though external evidence of chlamydospores or mycelium is frequently lacking. Though these spikes, cleared and stained, are seen to be permeated with mycelium to the very tips of the awns, the amount of mycelium is unquestionably much less than is found in normal smutted spikes, in which a spike consists of little host tissue other than a rachis on which masses of mycelium rounded off into chlamydospores are aggregated into sori. It seems, therefore, that the reduced chlamydospore formation at the high temperature is due largely to the direct effect of temperature in reducing the growth and vigor of the fungus.

Table 1.—Summary of results of studies of loose smut in wheat and barley at 3 greenhouse temperatures.[a]

65°F	75°F	85°F
Wheat		
Normal smutted spikes produced; smut infection of leaves in some varieties; failure of peduncles to elongate in some smut-infected plants of one variety.	Normal smutted spikes produced; reduced sorus and chlamydospore formation in some smut-infected spikes of some varieties. No smut infection of leaves.	Slender smut-infected spikes with few to no chlamydospores produced by 5–7 days or longer at 85°F, between early internode elongation and spike emergence; glumes, lemmas, and paleae often formed on infected spikes but other floral parts usually abortive; color of spike an uneven light green.
Barley		
Normal smutted spikes produced; some smut infection of leaves.	Normal smutted spikes produced; soros development and chlamydospore formation partially or completely inhibited in some smut-infected spikes of some varieties; smut infection of leaves more pronounced than at 65°F and more extensive in some varieties than others; sori sometimes formed on peduncle and topmost node.	Slender smut-infected spikes with few to no chlamydospores produced by 7 or more days at 85°F, between the early internode elongation and the early boot stages of development; some infected spikes with almost completely bare rachises, others with single green sori replacing the groups of 3 spikelets; distinct glumes, lemmas, and paleae seldom formed, other floral parts usually abortive; chlorotic streaks instead of elongated black sori in smut-infected leaves.

[a] Based principally on the reactions of Ceres, Lee, and Marquis wheats, and Forrest, Montcalm, and Traill varieties of barley.

Table 2.—Average dry weight of mycelium of *Ustilago nuda* produced in 4 weeks at 6 different temperatures on enriched[a] potato-sucrose agar[b].

°F	Av. weight (g)
50	0.021
59	0.107
68	0.280
77	0.204
86	0.044
95	0.002

[a] Containing 400 g potatoes, 100 g sucrose, 1 g asparagine, 2.5 g yeast extract, 17 g agar per L.

[b] Based on 6–12 flasks at each temperature, the variation in number being due to contaminated flasks that were discarded.

That this high temperature effect can occur in the field is of practical importance and has many implications. If it occurs in the field, the hitherto accepted explanations for low loose smut in certain areas and under certain conditions may not be acceptable. The example of Freeman and Johnson (1)—a late planting of winter barley in Texas with a much lower percentage of loose smut than earlier plantings—could be explained on the basis that the late planting flowers late and at a higher temperature than the earlier plantings, a temperature high enough to inhibit chlamydospore formation and so mask the smut. Again, certain areas of the United States are notably free of loose smut, because, it is generally held, of low humidity at flowering time. To the contrary, the low incidence of smut could perhaps be attributed, in some cases at least, to inhibition of chlamydospore formation and consequent lack of inoculum.

The occurrence on infected barley plants grown at 75°F of spikes with normal sori, spikes with sori reduced in number and size, and spikes with no sori but with bare rachises, suggests the possibility of breeding barley varieties resistant to loose smut, using the inability to produce sori or the production of few sori by infected plants at moderate temperatures as an inherited character. Some evidence was obtained in the above experiments that varieties differ in the frequency with which they produce this bare rachis type of reaction and lines that consistently give this reaction could possibly be found. Incorporation of this character into commercial varieties would result in hypersensitive varieties that would be field immune to loose smut since no inoculum would be produced on infected spikes.

Embryo tests for loose smut usually give higher readings than field or greenhouse tests (6, 9, 10). The occurrence in the studies of spikes of wheat and spikelets of barley that appeared to be smut-free but had smut sori instead of ovaries, poses the question of the extent to which these infected spikes occur and are overlooked in loose smut nurseries. The discrepancies between embryo and field tests may be explained at least partly on this basis. Freeman and Johnson (1), in 1907, observed some spikelets of wheat in which only the ovary was infected. So there can be little doubt that some of the spikes that appear smut-free in field nurseries of loose smut, harbor minute sori on the glumes or within the florets.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul.

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PHYTOPATHOLOGICAL NOTES

SOIL TRANSMISSION OF TOBACCO RINGSPOT VIRUS

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Transmission of tobacco ringspot virus (TRSV) by grasshoppers was demonstrated by Walters (5) and Dunleavy (1). Since transmission percentage was low, grasshoppers are probably not important vectors of the virus (1). The common occurrence of TRSV in several hosts has led to an assumption that efficient vectors must exist, though none have been reported. Similarities to certain soil-borne ringspot viruses (2, 3) suggest that TRSV may also be soil-borne. Preliminary studies give positive evidence of the soil-borne nature of TRSV.

The virus used was isolated from a watermelon affected by the "pimples" disease (4). By its symptoms on several hosts, properties, and cross-protection against a known strain of TRSV (American Type Culture Collection No. 98) it was shown to be a yellow strain of TRSV.

Infestation of virus-free soils was attempted in several ways. Initially, autoclaved soil in 6-in. pots was treated with infectious cucumber sap (10 pots) or coarsely shredded infected cucumber plants (15 pots). Cucumber, sugar beet, or watermelon seeds were planted immediately after soil treatment. After 7 weeks the roots of the plants in each pot were indexed by inoculation to cucumber. No plants were infected.

Virus-free soil was successfully infested by growing infected plants in the soil. Model cucumber seeds were planted in 12 pots of soil from a northwestern Arkansas vineyard. When the plants reached the cotyledonary stage, half were inoculated with TRSV. Five weeks later, shoots of all plants were removed at the soil level and indexed. The soil was then removed from the pots, broken up, fertilized, and replanted with Model cucumber. About 30 days later the youngest enlarging leaves of the plants in 2 of 6 pots developed chlorotic spots, rings, and diamond markings typical of symptoms of TRSV. Mechanical inoculation from these plants confirmed the presence of TRSV.

Soil infestation was achieved again with 2 soil lots, one the same as above and the other from a northeastern Arkansas soybean field. Healthy and infected Charleston Gray watermelon plants were transplanted into 6-in. pots containing these soils. After 2 weeks the shoots of the watermelon plants were removed, and the soil in each pot was divided between two 4-in. pots, 1 pot being replanted to Black Diamond watermelon and the other to Alaska pea. Soil transmission occurred to pea in 1 pot of vineyard soil and to watermelon in 2 pots of vineyard soil and 1 pot of soybean soil.

In a third study, cucumber plants in 10 pots of autoclaved soil were inoculated 2 weeks after planting. *Xiphinema americanum*, American dagger nematodes (100 per pot), were placed in half the pots. The nematodes had been suspended in water several days and were inactive. At the end of the experiment only 1 of the 500 nematodes was recovered by the sieving–Baermann funnel method. Eight weeks after planting, the shoots of the cucumber plants were removed as in the other experiments and the pots replanted to cucumber. Five weeks later the shoots and roots of the second crop were indexed. Soil transmission occurred in 2 of the 5 pots of each group.

To test for natural soil infestation with TRSV, 5 soil lots were obtained in April, 1960, from a field where watermelon "pimples" disease incited by TRSV was prevalent in 1959. Each soil lot was obtained at random from a small area of the field, and all sections of the field were represented by the 5 lots. Healthy watermelon plants were transplanted into five 6-in. pots of each soil lot, and healthy cucumber, eggplant, sugar beet, and tobacco plants were transplanted into 1 pot each of each soil lot. In addition, 5 watermelon, 3 cucumber, 3 eggplant, 3 sugar beet, and 3 tobacco plants were transplanted into autoclaved soil. After 9 weeks the roots and shoot of each plant were indexed. TRSV was recovered from the roots and shoot of one watermelon plant and from the roots of 1 tobacco plant, both from the same soil lot.

Soil transmission of TRSV is not limited specifically to a few hosts; transmission occurred to cucumber, watermelon, tobacco, and pea. Transmission in 4 soils from different localities indicates that many soils are receptive to this virus.

Further studies will be required to determine the extent of soil transmission, the conditions under which it occurs, and the significance of soil transmission of TRSV in field development of the diseases it causes.—Department of Plant Pathology, University of Arkansas, Fayetteville.

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EFFECT OF AUXINS ON VIRUS-INDUCED LEAF ABSCISSION

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Based on an M.A. thesis, at Montana State University.

A number of plant viruses have been shown to induce premature leaf abscission in their hosts. These include tobacco mosaic virus (TMV) in *Physalis floridana* L. (6), *Browallia speciosa* Hook., and *Capsicum frutescens* L. (3), and potato virus Y in *Solanum tuberosum* L., *Physalis floridana* L., and *Lycium barbarum* L. (6).

Since a decline in the auxin content of leaves has long been claimed to be an important factor in causing leaf abscission (4), the question arises whether this is also the cause of the premature abscission associated with some virus infections. There is considerable evidence that virus infection lowers the auxin content of host plants (5), which would also agree with other symptoms of virus infections, such as witches' brooming (7), plant stunting and inhibition of root formation (2).

External applications of indole acetic acid (IAA) delayed leaf abscission in virus-Y-infected potato plants (1). To determine the generality of this phenomenon, we have studied a different virus-host combination, and have also used the synthetic auxin napthalene acetic acid (NAA) as well as IAA.

Seedlings of *P. floridana* grown in 4-in. pots in a loam soil were inoculated on a lower leaf with the common strain of TMV. Experiments were run between April and June 1958. Minimum greenhouse temperature was 18°C, but maximum daytime temperature varied with the season, occasionally reaching 35°C in the June experiment.

Beginning at the time of inoculation, plants were sprayed to runoff at 14-day intervals with aqueous solutions of varying concentrations of the potassium salts of IAA or NAA. A count was made at least twice weekly of the number of leaves abscissed.

In preliminary experiments concentrations of NAA above 100 ppm and of IAA above 1000 ppm produced much deformation of plants and were not used in later experiments.

There was considerable variation from one experiment to another, but definite trends are apparent when the numbers for a particular treatment were combined and averaged for the three experiments, as shown in Table 1.

The effects of NAA are consistent with the hypothesis that a decrease in auxin is responsible for the premature leaf abscission, since there is a direct proportionality between the log of the concentration of applied NAA and the degree to which the effects of virus are countered. However, the fact that higher concentrations of IAA, presumably the naturally occurring growth substance, actually speeds abscission, is at variance with this hypothesis.

Table 1.—The effect of napthalene acetic acid (NAA) or indoleacetic acid (IAA) sprays on leaf abscission of Physalis floridana infected with tobacco mosaic virus.

Growth substance concentration (ppm)	Av. no leaves abscissed per plant 47 days after inoculation in 3 trials with 154 plants	
	NAA	IAA
0	26	25
1	19	12
10	17	20
100	9	18
1000	0	34

Also, because of the abscission-speeding effect of the higher concentrations of IAA, NAA appears to be potentially more useful in delaying or preventing premature leaf abscission caused by virus infection in crops.—Department of Botany, Montana State University, Missoula.

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CHEMOTAXIS OF ZOOSPORES OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE BY PLANT ROOTS AND CERTAIN CHEMICAL SOLUTIONS

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Portion of an M.S. thesis, North Carolina State College.

The zoospores of *Phytophthora parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker, causal agent of the black shank disease of tobacco (*Nicotiana tabacum* L.), are the most important means by which this fungus spreads during the summer months. Hence, zoospore production potential, discharge, and motility are important considerations in studying the epiphytology of the black shank disease. During such a study, it was discovered that zoospores of this fungus were attracted en masse to wounded areas of roots from tobacco and other solanaceous plants. No report was found of this phenomenon or the influence of

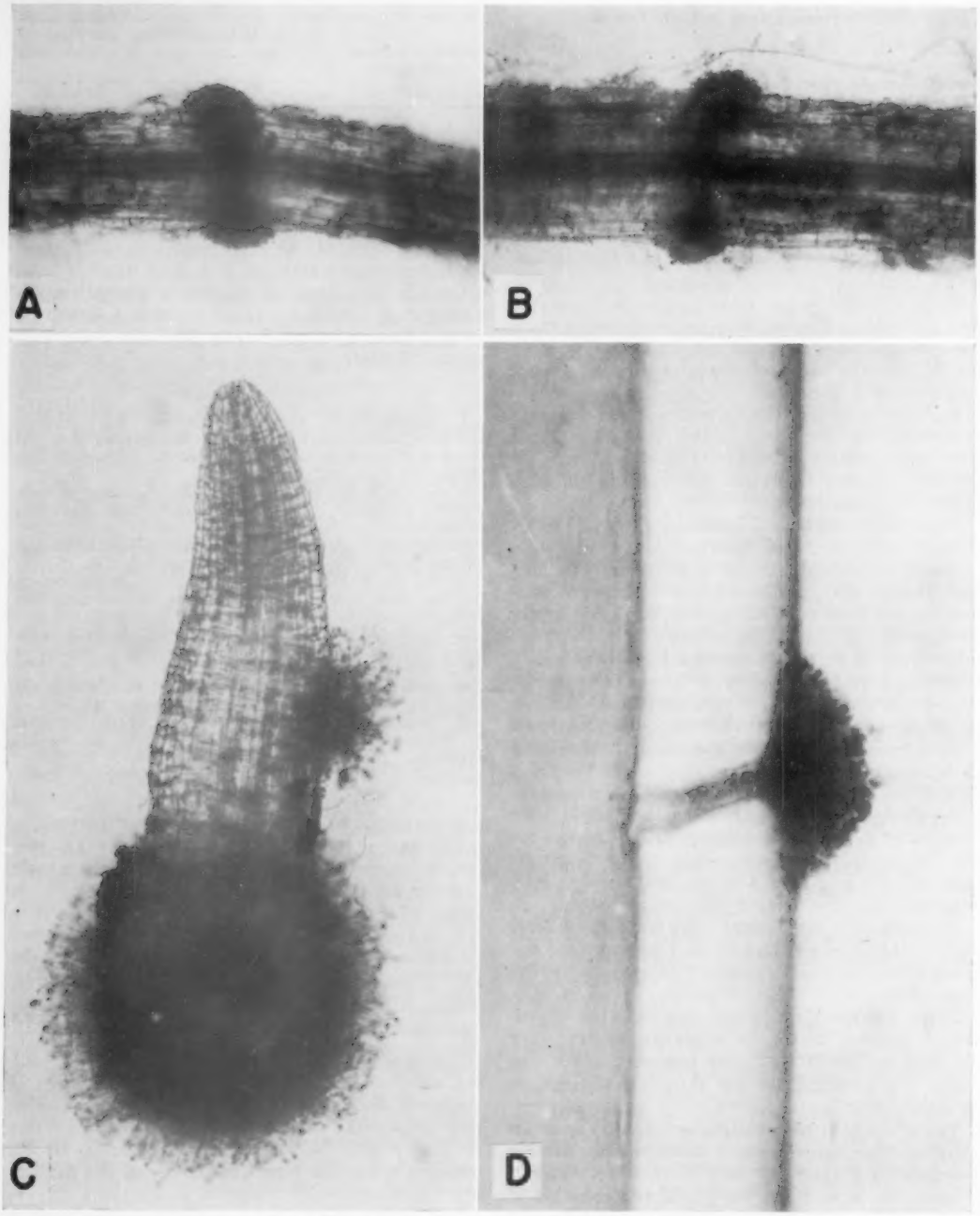

Fig. 1.—Attraction of zoospores of *Phytophthora parasitica* var. *nicotianae* by wounded plant roots and by a 1% sucrose solution. **A)** A live tobacco root showing a large mass of zoospores at cut area. Photograph taken about 2 hours after root placed in zoospore suspension (×100). **B)** Irish potato root (×100). **C)** Stained root of pepper showing a very large accumulation of zoospores at cut end and a similar massing at needle puncture (×200). **D)** Massing of zoospores at opening of a canal in a polyethylene tube containing 1% sucrose solution (×133).

plant roots on the motility and activity of zoospores from a phycomycete.

Freshly excised roots in a Syracuse watch dish containing an aqueous zoospore suspension were observed under the microscope. Attraction occurred in 1–10 minutes when the roots were partially submerged in the zoospore suspension. No attraction occurred if the roots settled to the bottom or remained on the surface of the suspending medium. Zoospore concentration affected the time required for massing on the roots; consequently, all subsequent tests were conducted with a standard dilution of 100,000 zoospores per ml. The optimum temperature for zoospore attraction to roots was 20–24°C, the same as for the maximum motility period and for germination.

Zoospores were attracted to tobacco roots from either a black-shank-resistant or a black-shank-susceptible variety. Susceptible and resistant host roots could not be distinguished by response time or the massing of the zoospores. Responses were similar with roots from *Nicotiana plumbaginifolia* Viv., a species with high resistance to black shank.

In addition, zoospores were attracted to roots of potato, pepper, and egg plant (Fig. 1) to the same degree as to tobacco roots. Repeated efforts failed to demonstrate attraction to tomato roots.

When the root epidermis was broken upon removal from soil or by girdling with a scalpel, the zoospores formed a band encircling the root (Fig. 1-A,B). A small puncture with a needle or small cut resulted in a mass of spores (Fig. 1-C). Fig. 1-A shows such a mass of live zoospores on a tobacco root 2 hours after the root was placed in the zoospore suspension. The other photomicrographs, of zoospores on pepper and potato roots, were made after the spores were stained.

Attempts to ascertain the substance or substances responsible required a technique whereby the test substance could be brought near the zoospores without agitating them during microscopic observation. This was done by placing the test solutions in a capillary polyethylene tube (inside diameter of .023 in.), sealing the open ends, and making small perforations in the wall with a steel needle. A 1% aqueous sucrose solution, which provided strong attraction (Fig. 1-D), was used as a standard. Several other sugars and casamino acid performed similarly to sucrose, but no attraction was observed with lactose, galactose, tap water, or NaCl (Table 1).

Chemotaxis of zoospores to plant roots is an unusual and interesting phenomenon. It may apply not only to *P. parasitica* var. *nicotianae* but to other soil pathogens that produce motile spores. The role of zoospore attraction in disease development is not known, and can be only the subject of conjecture at this time. However, this mechanism may play a very important role. It has been assumed that zoospores move at random in the soil and if by chance they come to rest in contact with a root of a favorable host, they encyst, germinate, penetrate the root, and result in disease. These studies demonstrate that zoospore movement is not entirely random. Since the functional distance of this attraction mechanism is unknown, its importance to disease incidence cannot be estimated.

Table 1.—Attraction of zoospores of *Phytophthora parasitica* var. *nicotianae* to various chemicals in aqueous solutions.[a]

Chemicals	No. times tested	Attraction rating[b]
Sucrose	10	+++
Sucrose (.5%)	5	+++
Dextrose	4	+++
Fructose	5	+++
Fructose (.5%)	5	+++
Rhamnose	5	+++
Maltose	6	+++
Maltose (.5%)	5	++
Casamino acid	4	+++
Casamino acid (.5%)	4	++
Arabinose	5	+
Ribose	5	+
Xylose	5	+
Galactose	5	0
Lactose	20	0
Lactose (.5%)	5	0
Tap water	4	0
NaCl	5	0

[a]All solutions tested at a 1% level unless otherwise indicated.

[b]Degree of attraction based on sucrose (1% solution) as the standard. Attraction rated as follows: Excellent, +++ (same as sucrose); medium, ++; weak, +; and none, 0.

The studies to determine the substance or substances responsible for this attraction do not prove that the solutions that gave a positive reaction with the polyethylene capillary tube technique are the same as those that cause attraction to roots. No doubt some of the chemicals tested are present in tobacco roots in sufficient quantity to cause chemotaxis.—Department of Plant Pathology, North Carolina State College, Raleigh.

CORN GRAY SPOT CAUSED BY PIRICULARIA GRISEA

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We are grateful to Dr. J. M. Waterston, Director of Agricultural Research, for advice, and to Dr. M. B. Ellis, Commonwealth Mycological Institute, Kew, for confirmation of the identity of this fungus. Material has been deposited in Herb. I.M.I. (Nos. 80978 and 80979).

Piricularia grisea (Cke) Sacc. has a wide host range among Gramineae (2), but has not previously been recorded on *Zea mays* L. Experimental infection of corn with *Piricularia*, in cross inoculation tests was reported with isolates from rice (1, 3) and *Eleusine coracana* (L.) Gaertn. and *Setaria italica* (L.) Beauv. (3).

In July, 1959, a leafspot disease was observed in a greenhouse at Ibadan, Western Nigeria, that was being used for the testing of varietal reaction of corn to *Puccinia polysora* Underw. A species of *Piricularia*

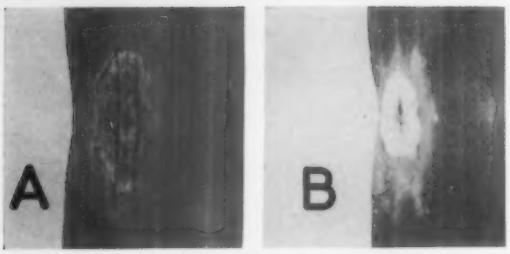

Fig. 1.—Corn gray spot on seedling greenhouse plants. **A)** Lesion on plant from rust assay greenhouse. **B)** Lesion produced by experimental inoculation. ×3

that was indistinguishable morphologically from *P. grisea*, was associated with the lesions. The leafspots were oval, up to 6×12 mm, gray to olive-gray, the older spots with a darker, powdery, sporulating center and an indistinct narrow brown margin (Fig. 1-A). The lesions often became confluent, leading to collapse of the whole leaf. Single-spore isolates were made before the fungus was eradicated from the greenhouse, where it has not reappeared.

In April, 1960, material was collected in the field from young plants also infected with *Piricularia*. The mature leafspots were round to oval, up to 5×8 mm, pale tan, with a light-brown margin often surrounded by a chlorotic halo, and a distinct dark gray sporulating center. Single-spore isolates from this material grew and sporulated well on Difco corn-meal agar, and young pot-grown plants of 3 varieties were infected with them. A macerated ten-day-old culture was sprayed onto the leaves after they had been gently rubbed between the finger and thumb to remove some of the waxy bloom. Controls were replicate plants sprayed with macerated sterile agar. The leafspots that developed after transfer of these plants to the greenhouse were at first similar to those observed on the rust-assay plants in 1959, and later came to resemble the spots seen in the field, with pale centers and brown margins (Fig. 1-B). *P. grisea* was reisolated from the inoculated plants. Rice plants were also inoculated with the same isolate with the same technique: no lesions developed.

Gray spot in the field was previously noticed and photographed in April, 1959, without critical examination. Specimens collected then have been found identical with the 1960 material. In 1959 the disease was severe enough to warrant scoring its intensity. The two lowest leaves of 20 4-week-old plants, chosen at random from each of six plots of 12 varieties, were examined and the number of lesions counted. Plot and variety scores were expressed as the mean number of lesions per leaf. The results are summarized in Table 1.

Corn gray spot is apparently more severe on young plants, and a test is intended with controlled inoculations of leaves of different ages. The low incidence recorded on the local variety Lagos White is in marked contrast to the high susceptibility this variety shows to other leaf pathogens, in particular *Puccinia polysora* (3). It is yet to be determined whether the Nigerian *P. grisea* is particularly pathogenic to corn or whether other factors predispose plants to infection. A common local weed grass, *Eleusine indica*, is heavily infected with *P. grisea*, and this may be a reservoir of inoculum, but preliminary cross-inoculations with the corn and *Eleusine* fungi have been unsuccessful.—Federal Department of Agricultural Research, Ibadan, Nigeria.

Table 1.—Mean number of *Piricularia* lesions per leaf on two lowest leaves of corn plants in April, 1959.

Variety	No. of lesions/leaf
Trinidad	46.2
White Tuxpan	19.3
Haiti	18.2
Mexico 7.	15.0
Sicaragua	14.0
Mexico 1.	12.2
EAFRO 231	10.4
Tsolo	9.6
Mexico 5.	9.3
Mexico 13.	8.7
Lagos White	5.6
Standard error of a mean	3.1

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PURIFICATION AND ELECTRON MICROSCOPY OF THE PEACH YELLOW BUD MOSAIC VIRUS

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Grateful ackowledgment is made to Dr. W. M. Stanley for making available the facilities of the Virus Laboratory, Berkeley, California.

An attempt was made to purify a sample of peach yellow bud mosaic virus (3) kindly provided by Dr. C. E. Yarwood, University of California. The hosts used were cowpea (leaves and cotyledons), French bean (leaves), and cucumber (leaves, cotyledons, and flowers). Success was achieved from cowpea leaves and cotyledons, using the butanol-chloroform method described by Steere (2). The aqueous supernatant resulting from this procedure was centrifuged at alternating high and low speeds (90 minutes at 40,000 rpm and 30 minutes at 7500 rpm, Spinco Rotor No. 40), finally yielding a light brownish but clear solution of high infectivity, despite the fact that some

infectious material was lost in the discarded pellet after each low-speed centrifugation.

The virus solution was then layered over a sucrose density gradient (layers of 13, 26, 35, and 45% sucrose allowed to stand in the centrifuge tubes overnight at 4°C), and centrifuged in the Spinco SW-39 rotor for 2 hours at 30,000 rpm. Three layers were seen in the centrifuge tubes under transmitted light: the top layer (about 12 mm below the meniscus) was light brown and slightly opalescent, the middle and lower ones (about 22 mm and 32 mm below the meniscus) were bluish-white and highly opalescent. The 3 layers were syringed off separately and dialyzed overnight against distilled water. They were then centrifuged for 3 hours at 40,000 rpm (Spinco rotor No. 40), the pellets resuspended in water, and finally centrifuged for 30 minutes at 10,000 rpm. The resulting supernatants were tested for infectivity by mechanical inoculation of cowpea, and the lesions counted. Both the top and middle layers from the sucrose density gradient were equally infectious, and were found to contain more or less spherical particles, 45–50 mμ in diameter (Fig. 1). This is in accord with the size and shape of particles found associated with other stone-fruit viruses (4). The bottom layer was not infectious and contained no such particles. No similar particles were found in any of the samples of healthy cowpea leaves and cotyledons processed in the same way.

Each of the 3 layers was centrifuged in the Spinco Model E analytical ultracentrifuge in 0.01*M* NaCl. The top layer showed 3 components: a small peak, $S_{20} = 113.4$; a major peak, $S_{20} = 76.2$; and a very small trailing peak, $S_{20} = 56.3$. The middle layer showed the presence of only one peak, not quite as high as the major peak of the top layer, with $S_{20} = 77.3$, so that these 2 major peaks were probably identical. The bottom, noninfectious layer showed no peaks.

Readings on the Beckman DU spectrophotometer in the ultraviolet range gave almost identical curves for the top and middle layers, although the O.D. of the middle layer was somewhat lower than that of the top layer, indicating, as did the analytical centrifuge, a lower concentration of material in the middle layer. The 260/280 mμ ratios for the top and middle layers were respectively 1.82 and 1.78, and the max./min. ratios were respectively 1.60 and 1.53. Whether the 260/280 mμ ratios reflect the presence of nonvirus impurities or the actual nucleic acid to protein ratio, is not known. Calculations based on phosphorus analyses and dry-weight determinations indicated a nucleic acid content of 8–10% as an upper limit.

Attempts to purify the virus from cucumber were partially successful with leaves (but not with flowers) with differential centrifugation in 0.01*M* PO_4 buffer, pH 7.0. The loss of infectivity as purification proceeded was considerable, but much less than when either water or PO_4 buffer of higher or lower pH was used as the suspending medium. In contrast to the results with cowpea, the use of butanol-chloroform gave noninfectious preparations. The virus solution from the differential centrifugation of cucumber leaves, when centrifuged through a sucrose density gradient, resulted, in different experiments, in a minimum of 4 and a maximum of 6 separate, visible layers. Unfortunately, all of the visible layers were infectious, to an apparently equal extent, and all had somewhat similar absorption spectra, although the 260/280 mμ ratios ranged from 1.4 to 1.9. In spite of the high infectivity in these layers, the preparations were obviously not highly purified.

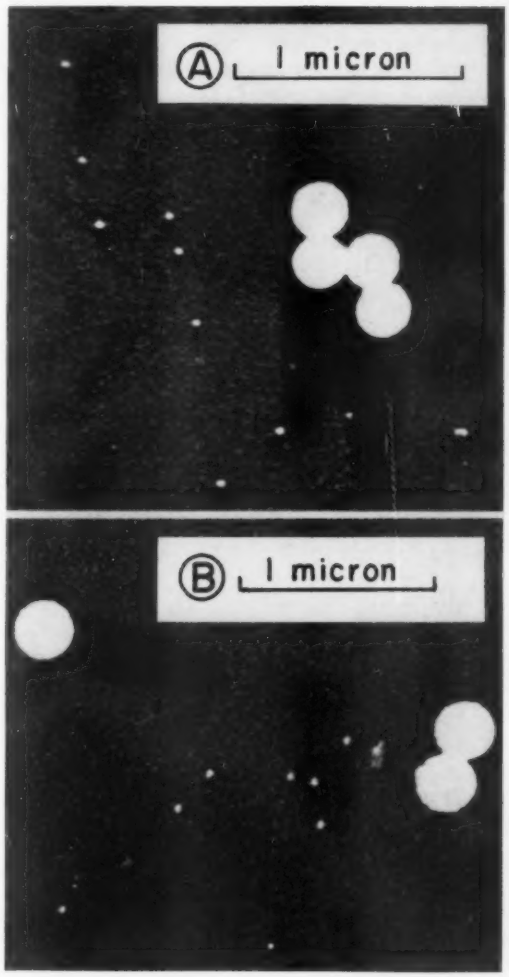

Fig. 1.—Particles associated with peach yellow bud mosaic virus purified by butanol-chloroform and sucrose density gradient centrifugation. **A)** Top layer. **B)** Middle layer from sucrose density gradient. The large white spheres are polystyrene latex particles, about 270 mμ diameter.

When French bean was used as the host, butanol-chloroform resulted in a total loss of infectivity and differential centrifugation yielded preparations with

very low infectivities. The use of the fluorocarbon Genetron (1) with French bean, cucumber, and cowpea resulted in total loss of infectivity.—6660 N.W. Marine Drive, Vancouver, B. C.

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SEROLOGICAL DETECTION OF BARLEY STRIPE MOSAIC VIRUS IN SINGLE SEEDS AND DEHYDRATED LEAF TISSUES

H. A. Scott

The Preer gel-precipitin technique (5) consists of layering warm liquid agar onto serum in small tubes and adding antigen after the agar has solidified. Both components diffuse into the agar, and the formation of a precipitate indicates a positive reaction between antigen and antibody. From the side of the tube the precipitate appears as a white band. This method, which permits study of antigen-antibody reactions of small amounts of materials, should be of value for detecting plant virus when tissue quantities are limited. The present investigation was conducted to determine whether a modified Preer technique would be applicable to the detection of virus in single seeds harvested from barley infected with barley stripe mosaic virus (BSMV), and in BSMV-infected leaf tissues desiccated for various periods (3).

Glass tubing with an inside diameter of 2 mm was cut into 5-cm lengths, coated with 0.1% agar in distilled water, and dried. Difco Bacto-Agar was made up at 0.6% concentration in 0.85% saline solution buffered to pH 7.2 with .01*M* phosphate buffer and autoclaved 20 minutes at 15 lb. Merthiolate was added to give a final concentration of 1:10,000. Agar (0.05 cc) was pipetted into the midpoint of each piece of glass tubing and allowed to solidify. In all tests, 0.05 cc of 1:10 dilutions of BSMV-antiserum [from Microbiological Associates, Bethesda, Maryland (1)], normal serum, and buffered saline were each placed on individual agar columns. Suspensions of healthy and diseased materials were placed on the other ends of the columns. All tubes were kept at 8°C in Petri dishes wrapped in moist toweling.

The seed coats were removed from Glacier barley seeds harvested from infected plants in 1953 and stored at cool temperatures for 6½ years. In germination tests about 64% of the seeds were found infected. Single seeds were ground with mortar and pestle and the pulp placed in 0.1 cc of buffered saline on individual agar columns. Since seeds from healthy Glacier plants were not available, virus-free Atsel barley and Rescue wheat seeds were subjected to identical treatment. The columns were examined daily for a white precipitin band signifying a positive antigen-antibody reaction.

White bands appeared within 24 hours in both healthy- and diseased-seed columns with antiserum and in normal serum and saline controls, suggesting a nonspecific reaction (Fig. 1-C,D). Bands from the saline controls contained protein rather than starch, as shown by lack of reaction with an I-KI starch test and a positive reaction with buffalo black (6).

Because nonspecific bands occurred in single-seed tests the precipitin bands located near the antigen source in the tubes could not be seen clearly. After the tubes were stored 2–3 weeks, however, the nonspecific bands were more uniformly distributed within the agar (Fig. 1-A) and the precipitin bands in the central area of the antiserum-diseased-seed columns could be seen (Fig. 1-B). In three tests with 60, 60, and 44 infected seeds, 20, 12, and 12 seeds, respectively, formed precipitin bands. Several agar columns were extruded from the tubes containing diseased material and the precipitin bands were removed and homogenized in distilled water. The homogenate was sprayed on screens, shadowed with chromium, and examined with the electron microscope. Short, straight rods were found like those described for BSMV (2).

Purification of the virus from infected seed resulted in the removal of the material responsible for nonspecific banding. One hundred infected and healthy seeds were ground in 40 cc of 0.2*M* phosphate buffer, pH 7.0, and centrifuged 30 minutes at 10,000 G. The supernatant was then centrifuged 90 minutes at 80,000 G. The resulting pellets were resuspended in buffered saline. Volumes of 0.05 cc of the final preparations were placed on several agar columns. Within 5 days precipitin bands were observed in the antiserum tubes containing the material from diseased seeds. Inoculation of 15 Atsel barley plants with the resuspended pellets from diseased seed resulted in infection of 12.

In the present study the modified Preer technique was readily applied to BSMV-infected barley and wheat leaves that had been desiccated and kept at about 1°C for 2 months to 7 years. Ten strains of BSMV were tested (4). The desiccated material was reconstituted by grinding it with mortar and pestle in 3 times its weight of buffered saline. The equivalent of 12 mg of dry tissue (0.05 cc of each solution) was placed on separate agar columns. Also included in these tests were saps from fresh, healthy tissues and from fresh tissues infected with 5 of the BSMV strains and reconstituted healthy barley and wheat leaves that had been desiccated for 3 months. The concentrations of antigen and antibody were such that all the diseased materials gave positive reactions within 2–5 days.

Fig. 1.—Agar columns showing specific and nonspecific precipitation. Upper portion of each tube contains pulp from a single seed. Antiserum or control solutions are below the agar. **A)** Virus-free Atsel barley seed vs antiserum after 20 days. **B)** BSMV-infected Glacier barley seed vs antiserum after 20 days. **C)** Virus-free Atsel barley seed vs antiserum after 24 hours. **D)** BSMV-infected Glacier barley seed vs buffered saline after 24 hours.

As an additional control, dehydrated Michigan Amber wheat leaves infected with brome mosaic virus were reconstituted and tested against BSMV antiserum and the homologous antiserum. Only the latter gave positive results. A negligible amount of nonspecifically precipitating materials diffused into the agar. No bands were formed in tubes containing normal serum or buffered saline.

An advantage in using desiccated virus-infected tissue for serological tests is that such tissue may be available at all times of the year and can be used as a positive antigen control when identifying unknown viruses.—Plant Virology Laboratory, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

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A TECHNIQUE FOR INOCULATING LEAVES WITH ROOT-KNOT NEMATODES

N. T. Powell and E. L. Moore

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Reliable inoculation techniques are necessary for critical research in plant pathology. For many pathogens, such as certain leaf-spotting organisms, suitable techniques have been perfected. For others, however, including some of the root-invading pathogens, exist-

ing inoculation techniques, although satisfactory, may in some cases need further refinement. Techniques involving root-knot nematodes (*Meloidogyne* spp.) fall into this latter group.

Methods currently in use for root-knot nematode inoculations usually involve growing the test material in infested soil. These methods permit contamination of the roots by other organisms and give results obtainable only by sacrifice of the plants inoculated. Foliar inoculation techniques, described by Linford (2) and used by Riggs and Winstead (4), were successful only when special humidity and other environmental conditions were provided to promote entry of the larvae. The need to keep the inoculated plants 48 hours in moist chambers further reduced the number that could be inoculated. The foliar inoculation described herein circumvents some of these disadvantages by employing a hypodermic syringe as used for stem and bulb nematodes (5).

Inoculum consisted of larvae of *M. incognita acrita* from roots of tomatoes (var. Rutgers). Mature egg masses were removed from the tomato roots with forceps and placed in Syracuse watch glasses each containing about 6 ml of distilled water. The glasses were left at room temperature until larvae had hatched from the eggs, usually in about 3 days. Distilled water was added periodically to prevent desiccation. After larvae had hatched, the larval suspensions from all the watch glasses were collected in a beaker. This final larval suspension was injected with a hypodermic syringe into the leaf midrib of flue-cured tobacco plants.

Selected for inoculation were plants 6–8 in. tall of root-knot-susceptible variety NC 400 (3) and root-knot-resistant breeding line Bel 6-31, in which root-knot resistance is dominant and monogenic (1). The inoculated plants were maintained in a greenhouse at about 80°F. Plants were also inoculated in a breeding nursery in early June.

Noticeable swellings were present on leaf midribs of susceptible NC 400 about 10 days after inoculation in the greenhouse. The swellings grew gradually, and well-developed galls were visible by the end of 4 weeks. Some smaller galls were present on lateral veins. Egg masses were produced in 5–6 weeks; some protruded through the epidermis and others remained embedded in the leaf tissue. No galls were noted on leaves of resistant Bel 6-31.

Results in limited field tests were similar to those in the greenhouse, though swellings were less prominent and could be detected only with difficulty.

This report is not intended to compare differences in reactions to nematode inoculation between resistant and susceptible host plants. Both types of plants were included to examine the possible utility of the foliar inoculation technique in screening tobacco plants for root-knot resistance. It is believed that such a procedure is especially suitable for greenhouse screening programs. In the field—especially with a crop such as tobacco, which produces abundant vegetative growth—gall formation may be masked by natural plant growth. The usefulness of this technique may be governed somewhat by the type of inheritance of resistance. For example, quantitative resistance may be more difficult to measure than simply inherited root-knot resistance. Also, the reaction to root-knot inoculation in foliage must be correlated with that in the root before one assumes them to be similar.

This technique is suitable for studying infection by root-knot nematodes and subsequent pathogenesis. It will enable investigators to determine precisely the time that entry occurs, and the various stages in parasitism can be accurately related to time. In addition, the loci of infections are known and may be studied before visible changes are evident.

Foliar inoculations provide a means of perpetuating pure cultures of root-knot nematodes. Leaf cultures are not as easily contaminated by other species as are root cultures. Frequent transfer of cultures would be necessary, however, because leaves of most plants become inactive rather quickly. Thus, the foliar inoculation technique may prove useful in certain phases of nematode research, depending on the nature of the problem and the host with which the investigator is concerned.

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RELATION OF TEMPERATURE TO REACTION TYPE OF PUCCINIA CORONATA ON CERTAIN OAT VARIETIES

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Waterhouse (8) found, in 1929, that temperature differences may affect some cereal rust reactions profoundly. Bromfield (2) recently reviewed the literature in this area. Peturson (5), in 1930, found the resistance to *Puccinia coronata* Cda. var. *avenae* Fras. & Led. present in 3 oat varieties at 57 and 70°F to be ineffective at 77°F. He further reported the moderate resistance of Red Rustproof at 57°F to his crown rust

Form 4 to be ineffective at 70 and 77°F although the reaction of Red Rustproof to his Form 3, highly resistant according to Murphy (4), was unchanged with temperature. Peturson also studied the effects of temperature alternation on the temperature-sensitive crown rust reactions. Observation by the present authors of a culture of race 263 of *P. coronata* var. *avenae* showing a differential reaction to the oat variety Glabrota upon change in temperature suggested the present investigation.

Seedling reactions of the oat varieties Canuck (CI 4024), Clinton 59 (CI 4259), Glabrota (CI 2630), and Saia (PI 186606) inoculated with 1 culture each of races 202, 263, and 295 of *P. coronata* var. *avenae* were determined at constant temperatures of 60, 70, and 80°F. Reactions of 26 additional varieties and lines to race 263 were determined at 60 and 80°F. Glabrota seedlings inoculated with race 263 and held at 60 and 80°F until full symptom expression were interchanged and observed for further symptom expression.

Between 60 and 80°F, higher incubation temperatures reduced the incubation period. Full symptom expression required 9 days at 80°F and 14 days at 60°F. The reaction type of Glabrota to race 263 varied from "0" at 60°F to a "1" type at 70°F, and finally to a "3" type reaction at 80°F (Table 1). The other 3 varieties did not respond this way, nor did Glabrota when inoculated with race 202 or 295. Reaction types of 26 additional varieties and lines to race 263 were not altered significantly with a temperature change from 60 to 80°F.

When Glabrota seedlings infected with race 263 were transferred from 80 to 60°F after full uredial development, a necrotic area developed around the initial "3" type pustule. Upon the reverse transfer, from 60 to 80°F, a ring of small secondary sporulating uredia developed on the periphery of the initial necrotic fleck.

The facts that Glabrota did not vary in reaction to all cultures with temperature change and that race 263 did not behave similarly on all resistant varieties indicate that the temperature response observed was not a general effect of temperature on either the resistance of Glabrota or the virulence of race 263. The variation in reaction must have been the effect of temperature change on the specific host-parasite interaction of Glabrota and the clone of race 263. This conclusion agrees with those of Johnson (3) and Bromfield (2) for similar occurrences with wheat and *P. graminis*.

The temperature-induced alteration of reaction of race 263 on Glabrota, coupled with the failure of alteration of the reaction types of races 202 and 295, also suggests either that at least 2 different genetic factors condition resistance of Glabrota or that there exists 1 factor which conditions resistance at low temperature but has a differential effect at high temperature.

Table 1.—The reaction types[a] of races 202, 263, and 295 of *Puccinia coronata* var. *avenae* on 4 oat varieties at 3 constant temperatures.

Oat variety	Race and temperature								
	202			263			295		
	60°F	70°F	80°F	60°F	70°F	80°F	60°F	70°F	80°F
Canuck	3[a]	3	3	3	3	3	3–	3–	3
Clinton 59	4	4	4	4	4	4	4	4	4
Glabrota	0	0	0	0	0–1	3	0	0	0
Saia	0	0	0	0	0	0	0	0	0

[a] 0 = no sporulation, highly resistant reaction; 0–1 = occasional minute pustules, resistant reaction; 3– = moderate sized pustules, moderately susceptible reaction; 3 = moderate to large pustules, susceptible reaction; 4 = large pustules, fully susceptible reaction.

The results from transfer between temperatures following initial symptom expression are similar to those reported by Bromfield (1, 2) and Silverman (7) with temperature-sensitive reactions of *P. graminis* var. *tritici*. Glabrota seedlings exhibiting a hypersensitive fleck at 60°F and yielding a ring of secondary uredia around the fleck following transfer to 80°F demonstrated that the rust mycelium in the hypersensitive fleck was still alive and could be induced to sporulate in the adjacent tissue by altering the temperature to produce a favorable host environment. A similar conclusion can be drawn from the "tissue transplant" technique reported by Sharp and Emge (6). This occurrence of inactivation rather than immediate death of the parasite is of particular interest in respect to the sequence of physiological events taking place in the hypersensitive reaction.—Purdue University, Lafayette, Indiana.

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SPORULATION OF PYRENOPHORA BROMI IN CULTURE

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A recently published note (6) asserted difficulty in isolating sporulating cultures of *Pyrenophora bromi* Died. and maintaining conidial sporulation on artificial media. We, in contrast, isolated sporulating *P. bromi* and obtained very satisfactory conidial production for inoculation of host plants by a method described in detail (2,3), and confirming general results of Chamberlain and Allison (4). Infection and symptom expression of *P. bromi* were excellent on several breeding lines of bromegrass in the greenhouse. See Fig. 1 of Carter and Dickson (3). The same brome lines were inoculated successfully in the field (3).

Eleven of 51 isolates studied in 1947-48 produced conidia profusely at first, but not continuously upon repeated transfer. Conidia-producing isolates became nonsporulating variably with time. Conidia-producing cultures were isolated from leaf blades as needed with no difficulty in 1949. Lesion-bearing leaf blades placed on moist filter paper in petri plates in the dark at 16°C produced conidia within 48 hours. Conidia were more abundant on blighted leaf tips or on large necrotic lesions than on small newly formed lesions. Conidia were sticky and were transferred aseptically, singly or in chains of 8-10, with freshly pulled glass needles under low-power binoculars. An apricot agar made with brome-leaf extract was used at first, but 1.7% soybean-meal agar, (100 g commercial soybean oilmeal, 1000 ml of water and 17 g of agar—oilmeal precooked in 500 ml of water for 30 minutes or suspended without precooking in 1.7% agar prior to pouring bottles and autoclaving) with or without brome-leaf extract, was very satisfactory for mycelial and conidial production. Temperature, light, and medium appeared to influence the amount of sporulation, so these factors were studied in 2 experiments in December, 1949.

Three cultures of *P. bromi*, 2 that produced conidia and perithecia and 1 only perithecia, were grown on 5 media in petri plates at 8 environments with 3 replicates (Fig. 1). Some contamination of the petri plates occurred at those environments requiring daily movement of the plates. Therefore, slants in 6-oz bottles were used in a second experiment with 5 media, 11 environments, and 2 replicates. Agar media used were soybean, soybean with brome-leaf extract, PDA, oatmeal, and apricot. Environments were (a to e) 8°, 16°, 24°, 28°, and 32°C in the dark; (f to h) 16°, 24°, and 28°C in temperature-controlled greenhouses with 12 hours of light and 12 of dark; (i) room temperature and variable diurnal laboratory light; (j) 12°C in dark for 8 hours, and room temperature in light for 16 hours; and (k) 12°C in dark for 16 hours, and room temperature in light for 8 hours. Petri plates and bottles were wrapped in thick black paper for all "dark" conditions.

Fourteen days after inoculation of bottles with conidia, data were taken on diameter of colony in mm, conidial production (1 = excellent, 5 = none), and production of immature perithecia (1 to 5 scale as above). Only conidia- and perithecia-producing isolate 2 and perithecia-producing isolate 3 were used in the second experiment, with bottle slants. Only data from bottles are reported in Table 1; these were analyzed statistically.

Media, environment and culture mean differences, and all interactions of main effects were highly significant except radial growth between isolate 2 and 3 in the 2nd experiment. These data suggested that many factors apparently control the manifestation of these characters or the degree to which they are expressed. Regarding light, Barnett and Lilly (1) found that bright light followed by a dark period, or continuous light of low intensity, was necessary for production of conidia by *Choanephora cucurbitarum* (Berk. & Rav.)

Table 1.—Diameter of mycelial colonies and production of conidia and immature perithecia by 2 isolates of *Pyrenophora bromi* in 11 environments, December, 1949 (Avg. of 10 observations—Temperature: C).

Culture	Dark					Diurnal light				12° Dark 8 hr	12° Dark 16 hr
no.	8°	16°	24°	28°	32°	16°	24°	28°	RT[d]	RT 16 hr	RT 8 hr
Colony diameter (mm)[a]											
2	14.1	24.3	30.8	20.2	20.3	31.5	33.4	25.8	39.0	24.7	23.9
3	16.4	33.6	35.4	12.0	8.2	34.7	28.1	20.8	35.3	31.2	30.9
Avg.	15.3	30.5	33.1	16.1	14.3	33.1	30.8	23.8	37.2	28.0	27.4
Conidial production[b]											
2	5.0	5.0	4.4	5.0	5.0	2.4	3.2	4.9	1.6	1.6	1.6
3	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Avg.	5.0	5.0	4.7	5.0	5.0	3.7	4.1	4.9	3.3	3.3	3.3
Perithecial production[c]											
2	3.0	1.0	2.0	2.8	2.6	1.4	1.8	2.6	2.0	2.3	2.0
3	4.2	2.3	3.1	3.0	3.9	2.7	3.0	2.5	2.7	2.7	3.2
Avg.	3.6	1.6	2.5	2.9	3.3	2.0	2.4	2.6	2.4	2.5	2.6

[a] LSD: culture x environment, 3.4 and 4.5; environmental averages, 2.4 and 3.1.
[b] LSD: culture x environment, 0.17 and 0.23; (environmental averages, 0.12 and 0.16, but average is not meaningful).
[c] LSD: culture x environment, 0.34 and 0.46; environmental averages, 0.25 and 0.33.
[d] RT, room temperature.

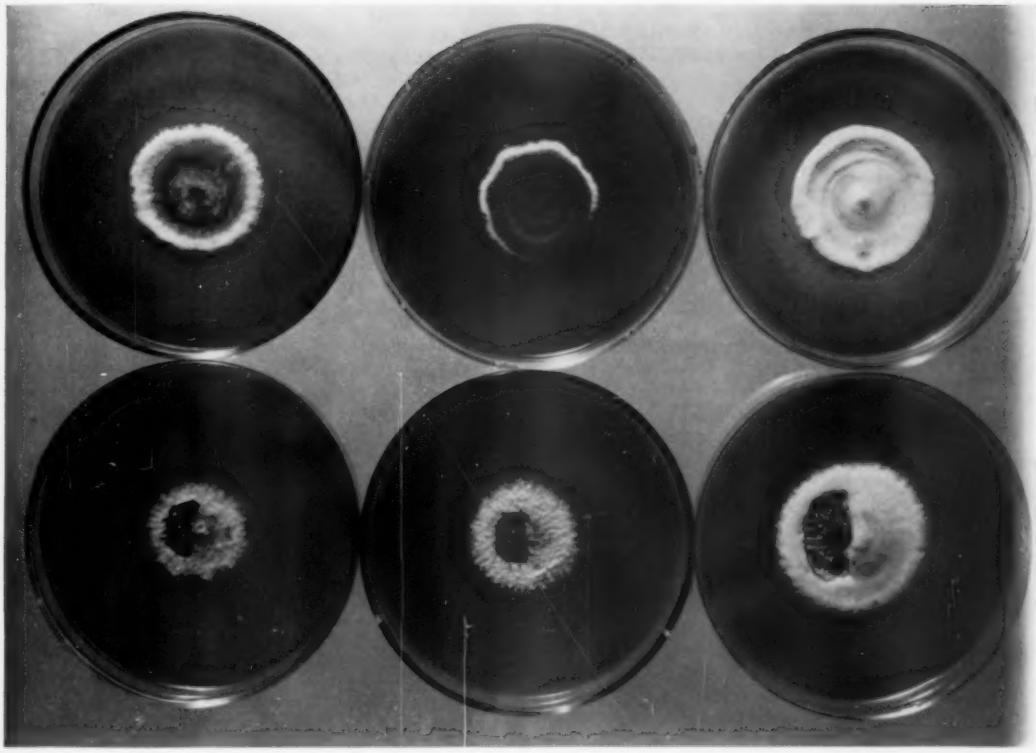

Fig. 1. Cultural characteristics on soybean agar of 3 isolates of *Pyrenophora bromi* that produced conidia and 1 that did not at 2 different environments. Left to right, cultures No. 1, 2, and 3; upper row 12°C and dark for 8 hours, alternate with room temperature and light for 16 hours; lower row—12°C constantly in dark, with the mycelium brushed down in a sector of the colonies to expose immature perithecia.

Thaxt. in culture. Richards (5) reported light necessary for sporulation of *Septoria nodorum* Berk.

Table 1 shows data on colony diameter, conidial producion, and perithecial production. The greatest radial colony growth was at 16°C or "room temperature" in diurnal light and 24°C in the dark. Significantly more radial growth occurred at 16°C with diurnal light than in the dark. Radial growth was least at 8°, 28°, and 32°C in constant darkness. The difference between cultures 2 and 3 in radial colony growth was nonsignificant over all treatments.

Environments f, i, j, and k resulted in highly significantly more conidial production by isolate 2 than at other environments. Conidia produced at 16°C appeared shorter but thicker-walled, more mature, and more uniform than conidia produced at other temperatures. Conidia were not produced in the dark on artificial media, except for a few at 24°C, possibly attributable to an accidental light leak. Conidial production was poor at 28°C in diurnal light. Isolate 3 produced no normal conidia, but at 28°C in diurnal light a few 2- or 3-celled deformed conidia (possibly conidiophores) were observed, giving a gray appearance to the colony instead of the color of conidia-producing colonies.

In both experiments, perithecial production was greatest at 16°C in the dark or in diurnal light. Fewest perithecia developed at 8°, 28°, and 32°C in the dark. Conidia-producing isolate 2 formed significantly more perithecia than isolate 3 at all environments except 28°C in the dark or in diurnal light. Perithecia produced by both isolates, examined microscopically, were immature and composed of starchy, granular, tetrahedral cells. Ascospores developed in these perithecia after 3-4 months of storage at 0-4°C supporting data of Chamberlain and Allison (4).

Colony diameter of isolate 2 and 3 was about 29 mm on soybean, soybean with brome extract, and oatmeal agar; and significantly greater than 24 mm and 20 mm, respectively, on PDA and apricot agar. The first three media were significantly superior to PDA and apricot agar for conidial production. More perithecia were produced on soybean plus brome-leaf extract agar than on any other medium, although PDA and apricot agar were nearly as efficient in perithecial production.

This study, though only qualitative, indicated media and environments useful for high conidial production by *P. bromi*. More precise experiments should reveal the exact conditions resulting in high conidial production.

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A WEED HOST OF XANTHOMONAS PHASEOLI VAR. SOJENSE

John P. Jones

Plant Pathologist, Crops Research Division, ARS, U.S.D.A. A report of cooperative work conducted by the Crops Research Division ARS, U.S.D.A.; and the Mississippi Agricultural Experiment Station, Delta Branch, Stoneville.

In September, 1959, plants of a common weed, "red vine" (*Brunnichia cirrhosa* Gaertn.), growing in a soybean (*Glycine max* (L.) Merr.) field at Stoneville, Miss., were observed heavily infected with a bacterial-type leaf spotting. Leaf symptoms consisted of small necrotic spots 1–2 mm in diameter surrounded by lemon-yellow halos 4–7 mm in diameter. Since soybeans growing in the field were heavily infected with the bacterial pustule disease caused by *Xanthomonas phaseoli* (E. F. Sm.) Dows. var. *sojense* (Hedges) Starr & Burk., the disease affecting red vine was studied further.

Inoculum was prepared from several red vine leaves in a manner previously reported (3). The inoculum was sprayed on leaves of greenhouse-grown plants of soybean varieties Arksoy, Clark, and Woods Yellow until the leaf tissue was lightly watersoaked. Check plants were similarly treated with sterile water. Heavy production of typical pustule symptoms occurred on the leaves of the three soybean varieties.

Isolations were made from individual pustules taken from the three varieties. Leaf segments were washed through several changes of sterile water, macerated, and diluted serially in nutrient dextrose agar. Typical *X. phaseoli* var. *sojense* colonies were recovered from all three varieties.

Inoculations were then made with the reisolated cultures to plants of soybean varieties Arksoy, Woods Yellow, and Lee. Abundant lesions typical of bacterial pustules were produced on Arksoy and Woods Yellow, but not on Lee. The negative reaction of Lee helped confirm the identity of the bacterium, since Lee possesses resistance to pustule.

Further inoculations were made to greenhouse-grown plants of red vine and Woods Yellow soybean with inoculum prepared from cultures of reisolated bacteria. Pustules were produced on both soybean and red vine leaves. In addition, a narrow chlorotic halo surrounded each pustule on the red vine leaves. What appeared to be *X. phaseoli* var. *sojense* colonies were isolated from the red vine pustules. Twenty-four days after inoculation, seven pustule-bearing red vine leaves were prepared as inoculum (3) and sprayed on Woods Yellow soybean leaves. Typical pustule symptoms resulted.

A final inoculation of red vine leaves was made, using two lots of inoculum, one consisting of soybean leaves naturally diseased with pustule, and the other of cultures reisolated from red vine pustules. Pustules were again produced by both inocula. Furthermore, a broad lemon-yellow halo, such as observed in the field, occurred around each pustule. The lack of broad halos in the previous inoculation was believed due to prevailing lower temperatures. In the final test, the temperatures were higher and more nearly approximated the conditions under which the symptom occurred in the field.

It is concluded from these data that *B. cirrhosa* is a natural host for *X. phaseoli* var. *sojense*. Only one species, *Dolichos biflorus* L., in addition to soybean has been reported as a natural host; *Phaseolus vulgaris* L., *P. lunatus* L., and *Dolichos lablab* L., however, have been infected artificially (1,2,4,5). All these species are in the Leguminosae, whereas *B. cirrhosa* is in the Polygonaceae.

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THE PRESIDENT'S COLUMN

Data are now available on an analysis of the membership as listed in the last Directory (Phytopathology 48: No. 5, Section 2, 1958). Sources of Employment and Professions are given in the accompanying tables. Fields of Interest will appear in a later Column. Similar methods of classification have been followed as explained in the first Directory (Phytopathology 43: No. 5, Section 2, 1953). Some 1,640 members were listed in the first Directory, while 2,018 appear in the current one. Since all members do not return the detailed information cards, the analysis that can be made in any one table will be less than the total membership. It is not believed, however, that this discrepancy will appreciably affect the comparisons or general conclusions.

The categories for Sources of Employment have changed but little relative to one another. Some 61% of the members are employed by state colleges and departments of agriculture. This is an increase of 3% over 1953. Included here are 191 research assistants and graduate students. Industrial positions continue to account for 14% of the membership. Federal positions have dropped from about 14 to 11%. In 1953, there were 19 self-employed; now we have only 6. While the number of industrial members has remained at the same percentage level, cognizance of this important group of our membership has been recognized by setting up a Standing Committee on Industry which is included among the basic committees of the Society structure.

TABLE 1.—Sources of employment for members of the Society in the United States and Territories

Classification	No. of members
State	896
Industrial	209
Federal	164
Federal and state cooperating	83
Endowed colleges, institutions, and foundations	68
Retired	42
Self-employed	6

In the list of Professions and Occupations, 62 different ones are recorded as compared to 42 in 1953. As before, administrative and also industrial titles (exclusive of strictly scientific designations) tend to vary considerably. Hence, there has been some grouping under what is believed to be an appropriate designation. This is indicated by the appended "etc."

The plant pathologists which include graduate students in departments of plant pathology, remain at an approximately constant level of 70% of our membership. The botanists, agronomists, mycologists, and biologists in 1953, totaling 127 members, constituted 10% of the membership. In the latest tabulation, the number remains essentially constant at 129 but this now represents only 7% of the membership.

The most notable change is the number of nematologists from 12 to 35, or from 1 to 2% of the membership. This of course reflects the rapidly growing emphasis on the study of nematode diseases.

TABLE 2.—Professions or occupations of members

Plant Pathologist	1220
Botanist	58
Research Administrator (state, Federal, endowed)	36
Nematologist	35
Research Administrator (industrial)	31
Agronomist	26
Mycologist	24
Biologist	22
Manager (industrial)	22
Agricultural Administrator, etc. (nonresearch)	21
Entomologist	21
Agriculturist, Experimentalist, Technologist, etc.	19
Horticulturist	19
Chemist, Biochemist	17
Laboratory Aide, Technician, etc.	15
Agricultural or Field Specialist (industrial)	13
Microbiologist	11
Plant Breeder, Geneticist	11
Plant Quarantine Inspector	11
Supervisor (industrial)	10
Executive Vice-President or Secretary, etc.	8
Plant Physiologist	8
College Dean, Consultant, County Agent, Group Leader, Technical Sales Representative—each	5
Bacteriologist, Collaborator Olericulturist, Industrial Representative—each	4
Dendrologist, etc., Pomologist, Superintendent Field Station—each	3
Agricultural Adviser, Biometrician, Biophysicist, College or University President, Curator, Editor, Food and Agricultural Officer, Science Teacher, Superintendent Field Station, Technical Adviser, Virologist—each	2
College Provost, Electron Microscopist, Engineer, Farm Adviser, Farmer, Medical Research Specialist, Soil Conservationist, Staff Associate, Viticulturist—each	1

Two professions very closely related to plant pathology have very small representation, notably plant physiology and bacteriology. In 1953 we had 7 and 2 members, respectively; the new count is 8 and 4. It would be desirable to broaden the scope of our Society and the basic approach of PHYTOPATHOLOGY to interest more of these scientists, all to our mutual advantage. We note that microbiologists have increased from 5 to 11 which is a favorable sign. "Olericulturist" is a good word readily found in the dictionary but apparently used only in California. We have included two Eastern specialists in vegetable gardening under this title and we hope they remain our friends.

Administrators—research, agricultural, and college (but not heads of departments)—together with industrial managers, supervisors, and executives account for 8% of our membership. No doubt many of these are successful and loyal plant pathologists. Others of these administrators, including a number of chemists, also have found the Society and its Journal rewarding. Let us continue to interest them and attract more.

S.E.A. McC.

NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

Virologist R. F. Stouffer was recently made Assistant Virologist at the Plant Virus Laboratory, University of Florida, Gainesville. Dr. Stouffer will continue basic research in plant virology.

Pathologist R. H. Porter is spending an academic year in Iran as adviser and consultant to the College of Agriculture in Shiraz. Dr. Porter's assignment was made possible by a Ford Foundation Grant. He will return to his home in Fort Collins, Colorado, in July.

Forest Nematologist John L. Ruehle, with the U. S. Forest Service, has joined the staff of the Department of Plant Pathology and Plant Breeding, University of Georgia, Athens. Dr. Ruehle recently received his Ph.D. degree at North Carolina State College, Raleigh.

The Southern Division held its annual meeting in conjunction with the Association of Southern Agricultural Workers, February 6-8 at Jackson, Mississippi. Approximately 90 members and guests attended the presentation of 36 papers and an informal smoker. Officers for 1961 are: President, A. L. Harrison; Vice-President, D. E. Ellis; Councilor, W. J. Martin; Secretary-Treasurer, J. P. Fulton. The next meeting will be held in conjunction with the annual meeting of the Society in Biloxi, Mississippi.

Nematologist L. R. Krusberg, Department of Botany, University of Maryland, College Park, has received a $9,700 grant from the National Institute of Health for studies on the physiology and nutrition of plant parasitic nematodes.

Pathologist E. W. Buxton, Rothamsted Experimental Station, visited the Pennsylvania State University and presented a seminar entitled "Variation in Fusarium."

Professor Clair A. Brown, Department of Botany, Louisiana State University, recently published a book on "Paleontological Techniques." The publication is a handbook for geologists and botanists on techniques of studying fossil pollens and spores.

Phytopathologist K. W. Kreitlow presented an invitational paper "Diseases of range forage plants and their effect on range management" at the annual meeting of the American Society of Range Management held in Salt Lake City, Utah, January 31-February 3.

Biophysicist J. T. Lett, with the Beatty Cancer Institute, London, and an expert in radiation biology, is spending a year's sabbatical leave on the staff of the Department of Plant Pathology, University of California, Berkeley. He is collaborating with Professor William N. Takahashi on studying virus protein synthesis under a grant from the U. S. Public Health Service.

Visiting pathologist G. J. Saaltink of the Laboratorium voor Phytopathologie, Wageningen, the Netherlands, will be studying wilt diseases at the Connecticut Agricultural Experiment Station, New Haven, during 1961. His visit to the New Haven Station is supported by a N.A.T.O. research fellowship awarded by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

All manuscripts submitted for publication in PHYTOPATHOLOGY should be sent to the new Editor-in-Chief, Robert W. Fulton, Plant Pathology Department, Madison, Wisconsin.

The Yale School of Forestry announces a notable expansion in its program of graduate instruction and research in Forest Pathology. Training offered at the doctoral level will have greater emphasis on physiological and biochemical aspects of forest disease and wood products deterioration problems than heretofore has been available in the United States. At the same time, the conventional program in the descriptive phases of the subject will be maintained, especially at the Master's level. To initiate this new program, the School has appointed Dr. Ellis B. Cowling as Assistant Professor of Forest Pathology, to succeed Dr. J. S. Boyce who retired as Professor Emeritus in 1958. Facilities for instruction and research in physiological as well as descriptive aspects of forest pathology are being installed in the School's new Greeley Memorial Laboratory. Nearly 10,000 acres of School forests are available for field work in three different forest types in New England. The program will be administered by the School of Forestry, in cooperation with the Departments of Botany, Microbiology, and Biochemistry at Yale University, with the Connecticut Agricultural Experiment Station, and the Forest Insect and Disease Laboratory of the Northeastern Forest Experiment Station of the U. S. Forest Service. Dr. Cowling holds B.S. and M.S. degrees from the University of New York College of Forestry at Syracuse University, a Ph.D. in plant pathology and biochemistry from the University of Wisconsin, and a "Filosofie Licensiat" degree in physiological botany from the University of Uppsala, Uppsala, Sweden. His experience includes a three-year appointment as pathologist at the U. S. Forest Products Laboratory at Madison, Wisconsin, and one year as a U. S. Public Health Service postdoctoral fellow at the University of Stockholm and the Biochemical Institute of the University of Uppsala.

Congratulations to M. E. Gallegly, Jr., professor of plant pathology at West Virginia University, who won the $1500 AAAS Campbell Award for Vegetable Research. The Campbell Soup Company established the award 3 years ago to recognize research of fundamental or practical significance to the production of vegetables for processing purposes. Dr. Gallegly was honored for his work on late blight in tomatoes and potatoes, which has made it possible to breed resistant strains. His contributions, made in cooperation with his associates, especially the staff members of the Rockefeller Foundation in Mexico, have been fundamental to a better understanding of the interaction of the host and pathogen in the disease incited by *Phytophthora infestans*. Dr. Gallegly has been at the West Virginia University since 1949.

Pathologist K. F. Baker, Department of Plant Pathology, University of California, has transferred from Los Angeles to the Berkeley campus. Beginning in March, under a Fulbright grant, he will be on a year's sabbatical leave in the Department of Plant Pathology, Waite Agricultural Research Institute, Adelaide, South Australia.

Deaths—Oliver F. Smith died August 21 at the age of 53. Dr. Smith had been employed continuously by the Forage and Range Research Branch, ARS, USDA, since 1934. During the first six years of this 26-year period of employment he worked cooperatively with the University of Wisconsin at Madison on the powdery mildew problem in red clover. The last 20 years he had been stationed at the University of Nevada at Reno. There he served successively in the capacity of plant pathologist and research agronomist, devoting full-time to research on alfalfa breeding and improvement. Dr. Smith received the USDA Superior Service Award in May, 1957, for outstanding contributions to agriculture in the United States.